A CHEMICAL AND HISTOLOGICAL STUDY
OF THE FEATHER PIGMENTS OF
THE DOMESTIC FOWL[1]

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MANY studies have been conducted in an effort to determine the intermediate reactions initiated by the discrete genes which culminate in the phenotypic expression. Much of such investigation has been concerned with the development of pigments in various organisms. The choice of color for studies of this type is probably due to the great number of variations and particularly to the striking differences observed. Some workers also consider the path between the initial gene action and the observed color to be relatively direct, which may or may not be true.

Pigment substances are very stable compounds which do not lend themselves readily to chemical analysis. Due to this fact, knowledge of the chemical and physical nature of the pigments is very limited. Progress in the attack on the general problem of pigmentation would be expedited by any additions to our knowledge of the nature of the end products of gene action. With this fact in mind, the following investigation was initiated with the intent of further clarifying the nature of the end products involved in producing the color patterns of the fowl. Using the feathers from a large number of breeds and

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varieties of poultry, a survey was made in which histo-
logical, spectrophotometric, and solubility observations
were recorded and analyzed.

Histological Studies of Feather Pigments
Material and Methods

Feathers from 37 different breeds and varieties of poul-
try, including red, black, blue, buff, white and various
gold and silver color patterns, were examined.   In order
to have more uniform samples, cushion feathers were util-
ized in most cases.   In a few instances, however, feathers
from other regions were studied if they showed coloration
which seemed to merit consideration.   The feather sam-
ples were supplied by specialized breeders in this country
and Canada.

Whole mounts of web and fluff barbules were prepared,
in order to study the distribution of the pigment and its
granular nature in the natural condition.   The desired
portion of the feather was placed in xylol for clearing.
This process required several weeks for the barbs but
since no medullary air spaces were present in the bar-
bules, they were completely cleared in only a few days.
Several more days were required for infiltration with
balsam.   In order to obtain a satisfactory lateral view
of the barbules they were removed from the barbs.   This
was accomplished by placing balsam infiltrated barbs on
a slide under a binocular microscope and carefully sever-
ing them at their bases by using a razor blade.   More
balsam was then added and a cover glass applied.

Cross sections of feathers in general proved much less
satisfactory for a study of feather pigments than the
whole mounts.   Many such sections were prepared and
some observations were made from them.   The samples
for sectioning were thoroughly cleared in toluene, the
time required ranging up to six weeks to penetrate the
comparatively thick barbs.   This was followed by infil-
tration with paraffin (m.p. 56–58° C.) in an oven held at
60° C.   They were then imbedded in hard paraffin (m.p.

60–62° C.).   The paraffin blocks were sectioned at three, five and ten microns after which the sections were mounted in balsam.

Liberated pigment granules were excellent material for determining the size, shape and uniformity of these bodies.   Liberation was accomplished by boiling the feathers for two hours in 6N HCl.   Longer treatment as suggested by Einsele (1937) did not appear to make the outlines more distinct and was therefore abandoned in favor of the shorter time.   In black feathers the keratin was destroyed and the granules became free in the solution.   No effect on the granules was noted and the filtrate showed no traces of dissolved pigment.   In red feathers, a part of the pigment was dissolved by the acid. A granular pigment remained, however, which was not further affected, even on boiling for as long as two days. All the pigment of buff feathers dissolved quickly in the acid, so that the method could not be applied to this color. The washed granules from black and red feathers were smeared thinly on glass slides and mounted in balsam.

White Varieties

Feathers from white varieties of Orpington, Langshan, Wyandotte, Silkie, Minorca, Plymouth Rock, and Leghorn breeds were examined (Table 1).   Definite black pigment granules were observed in all cases.   The largest granules were observed in the fluff, being irregularly rod- to spherical-shaped and about 1.1 µ in diameter and from 0.5 µ to 2.0 µ in length (Fig. 14).   These granules occurred rather infrequently, either singly or in groups of two to six or occasionally more, and were located in the center of the barbule just proximal to the nodes.   The White Plymouth Rock feathers examined showed more of this type of granule than the other breeds studied.   Of more frequent occurrence were very minute spherical granules, along with a very few rod-shaped ones, ranging from 0.5 µ in diameter to the limit of visibility (Fig. 9).   These granules were largely confined to the base of the barbules

and the sides of the barb to which the barbules were attached. Occasionally single granules or groups of granules of this type were found in the nodes of the fluff. Such granules were found in both web and fluff barbules but were irregular in their occurrence. In some cases they were common in the fluff but rare in the web or vice versa, and frequently only one barbule out of a number showed such granules. The facts that the residue of acid hydrolysis of white feathers contain many of these granules and that they are plainly visible at the base of the barbules in cross sections of white feathers are added evidence of their existence. In a great number of cases where a few definite granules were found in the flat, proximal end of the barbule, a group of refractile bodies appearing somewhat like colorless granules could be observed (Fig. 9). These might correspond to the colorless refractile bodies described by Hamilton (1940) and others, as the first stage in the formation of pigment granules within the melanophores, or they might be artifacts produced by the structural arrangement of the feather. Harman and Case (1941) have also observed colorless granules in the hair of guinea pigs.

The fact that pigment occurs in white feathers is not surprising, since gray ticking or black flecking in standard bred recessive white breeds is quite common, despite vigorous negative selection. Lippincott (1921) found rod-shaped black pigment granules in the down barbules of White Plymouth Rock and White Wyandotte chicks. They did not occur in all individuals, but in some cases they became sufficiently intense, in the Plymouth Rocks especially, to cause a "smoky" down coloration, a condition which is not known to affect the whiteness of the adult plumage. Furthermore it is hardly conceivable that apparently functional melanophores would be produced by white breeds, as reported by Hamilton (1940), and still not deposit any pigment whatsoever in the feather.

Contrary to these observations are those of Willier and Rawles (1940) who found no pigment in white varieties
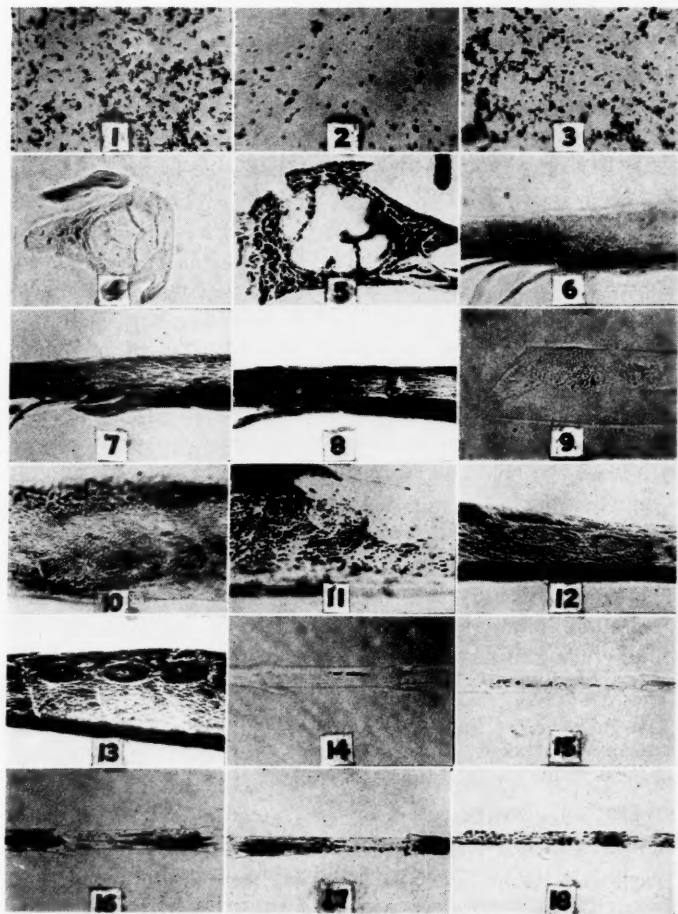
FIG. 1. Black Minorca, free pigment granules.   Fig. 2. Blue Andalusian, free pigment granules.   Fig. 3. Rhode Island Red, free pigment granules. Fig. 4. Buff Plymouth Rock, cross-section of barb.   Fig. 5. Rhode Island Red, cross-section of barb.   Fig. 6. Buff Minorca, distal web barbule.   Fig. 7. Red Leghorn, distal web barbule.   Fig. 8. Black Australorp, distal web barbule.   Fig. 9. White Plymouth Rock, proximal web barbule.   Fig. 10. Buff Minorca, proximal web barbule.   Fig. 11. Blue Andalusian, proximal web barbule.   Fig. 12. Red Leghorn, proximal web barbule.   Fig. 13. Black Australorp, proximal web barbule.   Fig. 14. White Plymouth Rock, fluff barbule. Fig. 15. Buff Minorca, fluff barbule.   Fig. 16. Blue Andalusian, fluff barbule. Fig. 17. Rhode Island Red, fluff barbule.   Fig. 18. Black Minorca, fluff barbule.

of the Wyandotte, Plymouth Rock and Silkie breeds as well as the Leghorn. They examined only fluff barbules where the writers found pigment less frequently than elsewhere in the plumage. They state that "usually" a complete absence of pigment granules exists, indicating that they may have found pigment in some cases.

Black Varieties

Black varieties examined included those of the Orpington, Australorp, Leghorn, Cochin, Ancona (Mottled), Minorca, and Andalusian breeds and black crossbreds showing gold in the hackle (Table 1). In all of these black specimens the granule shape and the distribution of pigment within the feather parts appeared to be the same. Any differences observed could be explained entirely on the basis of variation in concentration.

In the fluff barbules very uniform rod-shaped granules appeared (Fig. 18). These measured about 0.5 to 0.6 µ by 1.0 to 1.3 µ and were distributed in bead-like rows running longitudinally in the barbule. Only in the proximal region, near the attachment to the barb, was the arrangement disorganized. If only a small amount of pigment was present, the granules were localized in the distal half of the internode. As the density of pigmentation increased, the granules extended proportionally more toward the proximal end. Even in quite densely pigmented specimens, however, the concentration of pigment just proximal to the node could be observed. Usually the last places in the fluff barbule to show pigment were the nodes themselves. A marked dilution of the pigment in the nodes was evident in all cases except where extremely heavy general pigmentation was found in the barbule. This dilution at the nodes and the tendency to clump just proximal to the nodes gave such fluff barbules a distinctly segmented appearance when viewed under low power magnification.

Both the hooked and curved web barbules (Figs. 8, 13) were so densely pigmented that determination of granule

form was very difficult. The hooked barbules were uniformly and densely pigmented; the region distal to the hooks, however, appeared darker, probably due to the narrower and thicker structure in the region. They were always more densely pigmented than the curved barbules. The pigment also extended into the barbicels but only rarely did it extend the full length to the tiny hooklets or hamulae. Proceeding distally in the barbicels, the number of granules rapidly thinned until individual granules could be found and measured. The measurements here agreed with those in the fluff, the granules being characteristic rods about 0.5 μ in diameter by 1.3 μ in length, and invariably oriented longitudinally. In both hooked and curved barbules where pigmentation was very heavy, no cell boundaries or nuclei were seen. As pigmentation decreased, however, the granules were not deposited so close to the cell walls and pigment free areas indicated such boundaries. The nuclei of the original cells also became increasingly visible as the amount of pigment was reduced. Here the granules were densely applied to the nuclear wall, which gave the nuclei a distinctly oval appearance when viewed from the side. Near the cell boundaries rod-shaped granules could be observed, but elsewhere the barbules presented a "pebbly" appearance which some workers have interpreted as indicating round granules.

Acid extracted granules (Fig. 1) showed the same size and variation as those found within the feather. That no pigment had been lost during the process was indicated by the lack of any dissolved pigment in the filtrate from the acid hydrolysate. A light buff diffuse coloration was observed where the granules were concentrated within the feather. Although no color appeared in the filtrate from the acid hydrolysate, a similar effect as that seen in the feathers was observed wherever the granules tended to clump together in the acid insoluble residue. This is evidence that here at least, diffuse pigment as described by many earlier workers was not present.

Cross sections of the web from black feathers showed the pigment in the cortex of the barb to be distinctly stratified. These granules appeared round, as would be expected since the rods are placed longitudinally in the barb and a cross section of the rods was viewed. In the walls of the medullary cells, granules were also present and where a longitudinal plane was obtained they appeared rod-shaped. Pigmentation of the medulla was in no case observed inside of a cell cavity, but was always found within the keratinous walls of these cells. The barbules in such preparations showed no medullary cavities and the granules exhibited a uniform slightly oval appearance resulting from a diagonal section of the rod-shaped granules.

Willier and Rawles (1940) as well as Hamilton (1940) found black breeds of poultry to have rod-shaped pigment granules in the feathers. The latter described the melanophores of Jersey Black Giants as containing "long, thin rods with truncate ends" which were different from the short blunt rods of the Black Silkie melanophores. Lippincott (1921), however, found the rod-shaped granules of Black Andalusian, Orpington, and Langshan feathers to be identical.

Lloyd-Jones (1915) found two types of granules present in black pigeon feathers. One was a black rod, longer, more slender, and more variable in length than those found in the chicken. The other type was a sphere about the same size as that found in these studies in blue chicken feathers but which was not found in any black breed of poultry.

Blue Varieties

Blue Andalusians, Blue Cochins, Blue Splashed Andalusians and several blue crossbreds were utilized for the specimens of blue plumage (Table 1). In all cases where a blue color was exhibited, the granules were spherical and measured about 0.5 μ in diameter. In areas that appeared black, typical black rods occurred.

In the fluff barbules (Fig. 16) the spherical granules were usually found in bead-like rows oriented longitudinally, but frequently single granules could be found, and all were largely restricted to the distal half of the internode. In the most proximal area of the barbule the granules were more irregularly oriented and numerous spherical granules could be observed and measured. The Blue Cochin feathers were of a somewhat darker shade than the other breeds and showed a slightly more extensive pigment distribution, but no rod-like granules were found in the fluff.

The web barbules of the blues were unique in several ways. The granules were definitely spherical and appeared to be the same size as those in the fluff. Barbules from the periphery of the feather, however, showed typical black rods with the distribution typical of black granules. This accounts for the black lacing around the well marked blue feathers. The pigment was absent in the distal end of both kinds of barbules, the extent of the area devoid of pigment depending upon the shade of blue of the feather sample. The dark Blue Cochin barbules were virtually pigment free in the distal third, with only a few scattered granules remaining, while the lighter Andalusian had the pigment restricted to only the proximal third of the barbule (Fig. 11). Within the pigmented cells themselves, the pigment was restricted as compared with that in the black barbules. The positions of the nuclei of the cells were well defined due to a dense layer of pigment surrounding them. The other granules in the cell were grouped irregularly around the nucleus in the bead-like rows seemingly characteristic of round pigment granules. This left a relatively wide clear area between clumps of granules, but the cell wall was not well defined, since a few scattered granules or an occasional beaded string of granules extended entirely through the area. Nevertheless, a distinct cellular or segmental arrangement was noticeable even in the dark barbules of the Cochin.

Contrary to the situation in black feathers, the proximal barbules of blue feathers contained the most pigment. Here were found both a greater concentration of pigment within the cells and broader distribution of the granules. It would thus appear that the blue color of poultry is due to the combined effect of the shape of the granules and their restricted distribution within the individual cells, as well as in the barbule as a whole.

The residue from acid hydrolysis (Fig. 2) showed the same round granules seen in the feather and a few rod-shaped granules were found in the Andalusian, probably coming from the peripheral black barbules of the feather. More of these were found in the residue of Blue Cochin feathers where minute areas of black occurred in the web, giving the feather a stippled appearance.

These results are in complete accord with the earlier observations of Lippincott (1918) on blue chicken feathers. In pigeons, however, the situation seems to be somewhat different. According to Lloyd-Jones (1915) the granules although round, were two or more times the size of those found in the chicken. Also, in the pigeon barbs, the pigment was restricted to the medullary cells, the cortex being devoid of granules, while in the chicken, Lippincott (1918) described the uniform distribution of granules in both the cortex and the medullary cell walls.

Red Varieties

Feathers from Red Leghorns, New Hampshires, Rhode Island Reds, and Speckled Sussex were examined in studying the red pigment (Table 1). In red areas small spherical granules about $0.5\,\mu$ in diameter and of very uniform size were found. Another type of granule somewhat oval in character and measuring about $0.7\,\mu$ in diameter by $1.0\,\mu$ in length was found. The latter were far less numerous than the round granules and correspond in size, shape, and distribution to the granules found in buff feathers. In regions showing black pigmentation, only typical black rod-shaped granules were found. Their

distribution was also characteristic of black varieties but
the pigmentation was more dense than normally found in
true black breeds.   In the transitional areas both round
and rod-like granules were observable.   Ladebeck (1921),
Willier and Rawles (1940) and others, have described
round and rod-shaped granules in the Rhode Island Red.
No reference to the presence of oval-shaped granules
among the round granules has been made by anyone other
than Ladebeck, who considered them to be a variation
from the black granules.   The red pigment of pigeons is
also present as spherical granules according to Lloyd-
Jones (1915), but they are somewhat smaller being about
0.3 μ in diameter.

In the fluff barbules, the pigment was distributed
throughout the internodes (Fig. 17).   A tendency for the
granules to be concentrated in the distal half of the node,
and to be diluted or absent within the nodes, as was found
in other colors, was also observed here.   As the amount
of pigment increased the light nodal areas tended to be
obliterated except in the heavily pigmented fluff of the
main tail feathers where the almost pigment free nodes
provided a sharp contrast to the densely pigmented inter-
nodes.   Red areas showed both the small round granules
and the larger oval-shaped granules.   Where smut oc-
curred in otherwise red fluff, or in the gray fluff of the
Sussex, the granules were rod-shaped.   The small round
granules were densely distributed throughout the inter-
nodes in bead-like, longitudinally oriented rows.   The
oval granules where discernible, seemed to be localized in
the region immediately proximal to the node, the point
at which the pigment normally shows the greatest con-
centration.

In the web barbules (Figs. 7, 12) the distribution of
granules was similar to that in black feathers.   Granules
were of the spherical and oval types and were distributed
generally to all structures except the tips of the hooks.
Here again the hooked or distal barbules were more
densely pigmented than the curved or proximal ones

Pigmentation was also more dense in the proximal part of the barbules and when reduction in the amount of pigment was found, the loss occurred first in the barbicels, then the most distal portion of the barbule and receded proximally. In cases of reduced red pigmentation as found in New Hampshires or crosses between buff and red varieties, the loss seemed to be confined to the small round granules. The oval granules even seemed to increase in number, but this may have been due to the fact that some of them were masked by the round granules in the more densely pigmented feathers.

Determining the orientation of the densely distributed granules in the web was difficult, but here again it appeared that the round granules formed bead-like rows running longitudinally. The rows tended to be disorganized in the region of attachment to the barbs, and to curve around the position of the former cell nucleus, making its outline clearly distinguishable. They also tended to be absent from the area of the cell boundaries. This restriction was not so clean cut as was true in the case of black feathers since rows of granules frequently invaded the area and many single granules were scattered through it. Although apparently as densely distributed within the barbules as in the case of black feathers, they never became closely enough packed to present an opaque appearance such as was frequently found when black pigment was present. This would indicate that the density of color of the individual red granules is much less than that of blacks and that they therefore must be qualitatively different.

Residue from acid hydrolysis (Fig. 3) could not be expected to contain all of the pigment granules found in the feather sample, since some of the pigment was dissolved by the acid and appeared in the filtrate. However, a large number of the $0.5\,\mu$ spheres were found as were black granules, the number of the latter depending upon the presence and amount of black in the sample. They appeared exactly like those in the feather and it is un-

likely that the acid had affected them in any way. Notice-
ably absent from these preparations were the oval-shaped
granules although a few occurred if a large number of
feathers were treated for a short time with a small
amount of acid. Apparently, therefore, the oval-shaped
granules are the component which gives the coloring to
the acid filtrate of red feathers. Large amounts of the
so called "diffuse pigment" were observable in the
barbules wherever a quantity of pigment was present.
Since some of the red feather pigment was soluble in acid
it was impossible to determine whether diffuse pigment
as such was present in the feather, as contended by some
workers. However, since the reduction in the number of
oval granules could be correlated with the color of the
filtrate, it seems unlikely that diffuse pigment, as such,
was present. Furthermore, the same apparent diffusion
of pigment occurred in the residue wherever the granules
were clumped together as found in the feather.

Examinations of cross sections (Fig. 5) showed the
barb to contain much pigment in both the cortex and in
the walls of the medullary cells. The granules were in-
variably round as was expected and in the cortex showed
a distinctly stratified orientation. In the barbules, how-
ever, no definite arrangement was determinable.

Buff Varieties

Buff feathers from five breeds, Minorca, Leghorn,
Plymouth Rock, Cochin and Orpington were observed
(Table 1). The fluff barbules (Fig. 15) contained slightly
oval granules about $0.7\,\mu$ in diameter and $1.0\,\mu$ long.
These were placed in irregular rows but did not display
the bead-like arrangement exhibited by the round gran-
ules of blues and reds. Such granules were confined for
the most part to the distal half of the internode usually
with a definite clumping proximal to the node. Where
the granules were greatly concentrated, they extended
the full length of the internode and sometimes were even
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Pigmentation was also more dense in the proximal part of the barbules and when reduction in the amount of pigment was found, the loss occurred first in the barbicels, then the most distal portion of the barbule and receded proximally. In cases of reduced red pigmentation as found in New Hampshires or crosses between buff and red varieties, the loss seemed to be confined to the small round granules. The oval granules even seemed to increase in number, but this may have been due to the fact that some of them were masked by the round granules in the more densely pigmented feathers.

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In the web barbules the granules were not so numerous as in the fluff but retained their oval shape. They were more concentrated in the distal hooked barbules (Fig. 6) than in the proximal ones (Fig. 10) and although extending the full length of both types of barbules they were more concentrated in the proximal portions. In contrast to other cases of reduced pigmentation, such as exhibited by blue and light red feathers, a few granules were found in the barbicels. No definite arrangement into rows was observed as found in the fluff, most of the granules occurring as individuals. The lighter the shade of buff, the fewer granules found and the more restricted their distribution. A light buffish cast impregnated most of the barbules in the area surrounding the granules, being more intense near the greater concentrations of granules and fading to almost white at a distance. Some workers have described this as "diffuse" pigment and such a claim is difficult to disprove in buff varieties due, as in the reds, to the extreme solubility of the pigment in acid. However, this appearance could be explained as the result of refraction of light from the pigment granules, causing the surrounding keratin to acquire a shade corresponding to the pigment in the granules.

The granules from buff feathers were completely soluble in acid and none could be recovered in the residue after treating for two hours. However, by boiling a large number of feathers for a short time in a small amount of acid as was done with the reds, a few granules were obtained. These varied from normal sized oval granules to spheres of very small diameter. Due to the ready solubility of this pigment, it was difficult to determine whether the smaller granules normally occurred in the feather. Their presence in such preparations, however, was probably due to the partial dissolution of normal sized oval granules. It is possible to say definitely from this procedure that the oval-shaped bodies described in the feather were actually pigment granules and not artifacts.

Cross-sections of the web showed round appearing granules in both barb and barbules (Fig. 4). The barbules were heavily pigmented in comparison to the barbs. In the cortex of the latter the granules exhibited a stratified arrangement similar to the colors previously described, but were much more scattered. In the medullary cell walls, granules appeared round if viewed on end but definitely oval if a longitudinal plane was observed.

Ladebeck (1921) described a very finely granular to diffuse pigment of a yellow to yellowish brown color as occurring in the buff breed which he examined. Danforth (1937) found that the melanophores of Buff Leghorns contained granules smaller and more nearly spherical than blacks. These had a light yellow color and were somewhat soluble in fixing agents. The latter description, although indefinite, seems to fit the rather large granules described in this investigation. In pigeons the granules are so fine that Lloyd-Jones (1915) was unable to determine their size or shape. They occurred in formless clumps or agglomerations and he considered buff as merely an attenuated form of red. Such a condition evidently does not exist in chickens even on the basis of the description of Danforth where granules may be assumed to have a measurable size.

Silver Patterns

As examples of silver colored breeds, Columbian Plymouth Rocks, Light Sussex, and Light Brahmas were included (Table 1). The standard color description for females of this color pattern specifies black striping in the hackle and tail coverts and black in the main tail and wing feathers, the rest of the body feathers being white with "light bluish slate" undercolor.

The Columbian Plymouth Rock feathers examined were excellently marked, with a sharp line of demarcation between the white web and the gray fluff, the latter fading slightly toward the proximal end. The fluff barbules of this breed showed remarkably uniform rod-shaped gran-

ules of about 0.5 to 0.7 μ diameter and 1.0 to 1.3 μ length.
These were concentrated just proximal to the nodes
but were also found distributed somewhat less densely
throughout the rest of the internodes and the nodes as
well. The granules were oriented longitudinally to the
barbule and no exceptions to this arrangement were ob-
served. Wherever the granules were concentrated, as
near the nodes, a yellowish brown shading appeared
which might be considered as diffuse pigment by some
workers. Since this appearance was not observed about
any single granule it is doubtful that a diffuse pigment
as such is present. It is more likely that such an effect
is produced by the refraction of light by the grouped pig-
ment granules. Furthermore, no pigment was observed
in the filtrate from acid hydrolysis of such feathers, and
in microscopic preparations of the residue, wherever the
granules tended to clump together, an effect was pro-
duced as if diffuse pigment were present. If diffuse pig-
ment were present as such, it would be expected to appear
in the filtrate and not in the residue. Approximately
the same density of pigmentation was found in the web
barbules as was found in the average recessive white but
instead of being concentrated at the base, the pigment
was distributed throughout the entire length, the distal
barbules containing noticeably more granules than the
proximal ones. Occasionally granules were found in the
barbicels (hooks) themselves, even though there might be
none for some distance about them. The granules varied
from rod-shaped, as in the fluff, to spherical granules
varying from 0.5 μ in diameter down to the limits of visi-
bility. In the slides of acid hydrolysis residue, however,
the granules appeared to be almost entirely rod-shaped.
This may be explained on the basis of the observation of
Hamilton (1941) and Danforth (1937) that the shape of
the granules appears to be changed after ingestion into
the cells. In the present case, however, it may be that
the apparent change in shape is due to partial masking
by the keratin substance.

The Light Brahma and Light Sussex feathers were much too light in undercolor according to standard description, the gray being confined almost entirely to the rachis. The web barbules here were identical in pigmentation to those of the Columbian Plymouth Rock, although somewhat fewer granules were present. In the fluff barbules, however, the rod-shaped granules when present were concentrated in the distal third of the internodes with no pigment in the intervening spaces. As the amount of pigment increased, granules extended more proximally into the internodes, the nodes themselves being the last to be pigmented.

Other silver patterns examined included Silver Campines, Silver Penciled Plymouth Rocks and Barred Plymouth Rocks (Table 1). In the two former breeds, the granules and their distribution in both fluff and web barbs were typical of self (solid) blacks. In preparations of acid residues the granules were also characteristic rods as found in true blacks. In the fluff barbules of these varieties a lower concentration of pigment seemed to be necessary for the granules to pigment the nodes to an equal density as the internodes. In the white bands of the Campine the pigment was progressively reduced in each distally succeeding barbule. This was accomplished by eliminating pigmentation from the distal ends until a minimum in the barbules centrally located in the bands was reached. Then the proximal pigmentation gradually increased toward the next dark band. A few rod-shaped granules remained in all of the white barbules, and the light area could not be differentiated from barbules of white feathers. In the Silver Penciled Plymouth Rock, however, a large quantity of pigment remained in the distal half of the hooked barbules of the white area, while the curved barbules became almost colorless. The presence of so much pigment gave the penciled area of the sample feather a light dun appearance.

The granules of the Barred Plymouth Rock were slightly shorter on the average and somewhat more vari-

able in size than were those of normal blacks. Willier
and Rawles (1940) observed this same characteristic al-
though it seems to have been overlooked by Lippincott

TABLE 1

A Survey of Chicken Feather Pigments

Breeds	Apparent color of cushion feathers	Granule shape	Average granule size in microns	Solubility		Slope of log. E curve 500 to 700 milli- microns (× 100)
				Conc. HCl	0.5N NaOH	
Black Minorca	Black	Rods	0.5 × 1.3	−	+	− 0.3303
Black Orpington	Black	Rods	.5 × 1.3	−	+	− .3107
Black Australorp	Black	Rods	.5 × 1.3	−	+	− .3526
Black Cochin	Black	Rods	.5 × 1.3	−	+	− .3391
Black Leghorn	Black	Rods	.5 × 1.3	−	+	− .3335
Black Andalusian	Black	Rods	.5 × 1.3	−	+	− .3391
Barred Plymouth Rock	Black & White	Rods	0.5 × 1.0	−	+	− 0.3470
Columbian Plym. Rock	Black & White	Rods	.5 × 1.3	−	+	− .3224
Silver Campine	Black & White	Rods	.5 × 1.3	−	+	− .3224
Sil. P. Plym. Rock	Black & White	Rods	.5 × 1.3	−	+	− .3019
Ancona	Black & White	Rods	.5 × 1.3	−	+	− .3372
Blue Andalusian	Blue & Black	Spheres Rods	0.5 .5 × 1.3	−	+	− 0.3706
Blue Cochin	Blue & Black	Spheres Rods	.5 .5 × 1.3	−	+	− .3036
Gold Campine	Black & Buff	Rods Ovals	.5 × 1.3 .7 × 1.0	+	++	− .3497
Speckled Sussex	Red & Black	Rods Spheres	.5 × 1.3 .5	++	+++	− .5003
Brown Leghorn	Black & Brown	Varies		−	+	− .3487
Partridge Plym. Rock	Black & Red	Rods Ovals Spheres	.5 × 1.3 .7 × 1.0 .5	+	++	− .3401
Rhode Island Red Main Tail	Black & Red	Rods Spheres	.5 × 1.3 .5	++	+++	− .4763
Rhode Island Red	Red	Spheres Ovals	.5 .7 × 1.0	++	++++	− .6302
Red Leghorn	Red	Spheres Ovals	.5 .7 × 1.0	++	++++	− .6402
Buff Orpington	Buff	Ovals	0.7 × 1.0	+	+++	− 0.4481
Buff Minorca	Buff	Ovals	.7 × 1.0	+	+++	− .6198
Buff Cochin	Buff	Ovals	.7 × 1.0	+	+++	− .4089
Buff Plymouth Rock	Buff	Ovals	.7 × 1.0	+	+++	− .5406
Buff Leghorn	Buff	Ovals	.7 × 1.0	+	+++	− .5331
White Wyandotte	White	Varied		−		− 0.2518
White Langshan	White	Varied		−		− .3242
White Silkie	White	Varied		−		− .2620
White Plymouth Rock	White	Varied		−		− .3179
White Minorca	White	Varied		−		− .3372
White Leghorn	White	Varied		−		− .2749
White Orpington	White	Varied		−		− .2518

(1918). The average granule size was about 0.5 μ by
1.0 μ. They did not vary much in diameter but varied
considerably in length, occasional granules being as long

as normal black rods while a few others closely approached spherical proportions. Lippincott observed spherical granules only rarely in this variety. The concentration of pigment in the feather was in all cases more sparse than in black breeds. At the proximal edge of the white bar, the pigment free area began at the tip of the barbules and increased in successive barbules. In the middle of the white bar, only a few scattered granules were found in the barbules. If the bar was narrow, black pigment was evident in the tips of the barbules even before the proximal pigment had been entirely eliminated. More of the distal region of each succeeding barbule was pigmented until the completely pigmented barbules of the black band were reached. Thus no barbule was ever entirely devoid of pigment in both the tip and base at the same time, and the pattern of the bar on the individual barb was V-shaped. The light barbules of all three varieties were identical to those from white feathers and possessed many of the so-called colorless granules, some even occurring in the barbicels.

Gold Patterns

Gold color patterns examined included Gold Campine, Partridge Plymouth Rock and Brown Leghorn varieties (Table 1). In the Plymouth Rock and Campine the black areas had rod-shaped granules again typically black. The distribution of the rods was also typical of that in black breeds except that, as in the silver patterns, the fluff barbules required a lesser concentration of granules in order for the nodes to be pigmented. Nevertheless a distinctly segmental arrangement was maintained in all but the darkest fluff barbules. The light bars of the Campine feathers showed a buff coloration and the barbules appeared microscopically similar to those of buff feathers. The light bands of the Partridge Rock were more heavily pigmented and looked more like red pigmentation. However, the intense pigmentation of these barbules made it very difficult to determine whether a dilute

black or a red color was present. The fact that both feathers contained an acid soluble pigment indicated that some pigment other than black was present. Moreover, the acid hydrolysis residue of the Gold Campine feathers contained only black rods indicating that the oval-shaped buff granules must have gone into solution. The residue of Partridge Rock feathers, on the other hand, contained both typical black rods and round granules typical of red feathers.

The Brown Leghorn cushion feathers were unique in that the granules showed a great variation in size, ranging from rods about 1.5 μ long by 0.5 μ in diameter which are longer than black granules, down to almost spherical forms about 0.5 μ in diameter. The average of these lengths, however, was somewhat less than that of typical black rods. These variations were quite evident both in the acid residue and in the fluff barbules but none of these granules were acid soluble, indicating that probably no chemical difference exists between the different types. Whether the granules of the red areas of other regions of the plumage show different characteristics was not determined. Due to the density of the pigmentation and compact arrangement in the web barbules it was not determined definitely whether granules of a special size or shape were localized in the lighter-colored barbules. These lighter brown areas appeared, under the microscope, identical to the darker regions except that they were less concentrated.

Ladebeck (1921) found the feathers of the Brown Leghorn to contain variously shaped granules. He believed that all transitional forms from black to red were present. Furthermore, he believed that the intermediate color between black and red, such as found in the lighter areas of the cushion, consisted of granules which were transitional forms or ellipsoidal in shape. Although this situation might conceivably exist, it was not possible to demonstrate the fact by the techniques employed in this study, nor was the evidence sufficient to prove otherwise.

Discussion

Ladebeck (1921) stated that the distal barbules are more heavily pigmented than the proximal ones but he did not study the Blue Andalusian in which Lippincott (1918) found the reverse situation. The present investigation fully corroborates the observations of both authors. In the more extensive survey of chicken feather colors here recorded, in all colors except blue the distal barbules contained the most pigment. Lloyd-Jones (1915) described the distal barbules as uniformly containing the heaviest pigmentation in all colors of the pigeon.

In all cases where round granules were observed, they tended to be deposited in bead-like rows longitudinally oriented either in the barbule or barb. Black rods or oval-shaped granules were also uniformly arranged parallel to the long axis of the feather structure in which they were found. Only in a short region adjoining the barb was this precise organization varied, and here the arrangement was very irregular. These observations again confirm and extend the earlier work of Ladebeck (1921).

In the fluff barbules of all colors the distribution of pigment granules seemed to depend upon the density of the pigmentation. Where little pigmentation was present the granules were concentrated within a very limited area in the distal end of the internode. This suggests that the first pigment is laid down at this location in these barbules. Increases in the amount of pigment up to a certain point seem to result in greater concentration at this locus without any notable distribution to other areas. Further increments of granules result in pigment extending more and more proximally within the internode. In most cases a very dense pigmentation of the internodes was necessary before the pigment concentration in the distal portion was obliterated from view or granules were deposited within the nodes, which were the last regions to be pigmented. The required density within

the internodes which allowed the nodes to become pig-
mented, however, seemed to vary among varieties and
among sections on the same birds.   In Rhode Island Reds
the fluff of the main tail feathers showed extremely dense
pigmentation and the nodes were almost completely pig-
ment free.   In body feather fluff of the same bird a much
lower concentration was required to allow the nodes to
be equally as densely pigmented as the internodes.   Also
in Silver and Gold Campines and other breeds having
gold and silver patterns, the required density was not so
great as it was in the blacks.   Nevertheless, the general
rule as explained above seemed to hold for all colors.

Diffuse pigment has been described by many work-
ers as present in animals.   Included among these are
Haecker (1890) who observed diffuse pigment in red and
yellow bird feathers, and Ladebeck (1921) who described
it as present in a yellow variety of poultry.   Other work-
ers have staunchly denounced the idea of diffuse pigment.
As pointed out by Meirowsky (1912) and others later, the
so-called diffuse pigment does not occur except when
granules are present.   Esskuchen (1927) found diffuse
pigment associated with black and red cattle pigments,
more with the latter and less with the former.   Harman
and Case (1941) described red granules of guinea pig
hair as bright and translucent and associated with a large
amount of brilliant, orange-red diffuse pigment.   Choco-
late granules were darker and denser; and less diffuse
pigment, of a reddish hue, was found with them.   The
black granules were the most dense and were associated
with the least amount of diffuse pigment, that present
being a faint yellowish color.   This situation might be
explained by assuming that the diffuse pigment was a
degradation product of the granules and as a result of
this "chemical or physical reaction" with the keratin, the
pigment of the granules diffuses into the keratin, leaving
the granule more translucent and less dense than for-
merly.   The above authors seemed to favor this hypothe-
sis.   That such a hypothesis is untenable, in the case of

chicken feathers at least, is evident from the fact that Hamilton (1940, 1941) and Danforth (1937) and others have observed that the definitive refractive nature of the granules in the active melanophores has been determined before they come into contact with the keratin of the feather. However, a relationship similar to that described for the guinea pig by Harman and Case exists between the granular and apparently diffuse pigments of the chicken feathers, and therefore demands an explanation. The black feather granules were associated with a slight yellowish appearing diffuse coloration, the yellow feather granules with a more widely spreading diffuse coloration, and the red feather granules with a much deeper colored, apparently diffuse substance. It was further noticed that in no case did individual black granules show this phenomenon, its appearance being associated only with groups or clumps of granules.   The same situation only to a lesser degree was observed in red feathers, *i.e.,* fewer granules in one group were required for the appearance of the so-called bright red diffuse pigment. Just what the result would be if red pigment granules were as sparsely distributed as the granules of buff feathers is a matter of speculation only, as no such condition has been found. The granules of buff feathers were never sufficiently concentrated to form definite clumps, nevertheless, a yellowish cast in the keratin extended outward for some distance from only small groups of granules, becoming gradually lighter until the keratin again became colorless. On acid hydrolysis the feathers from, black, blue, and silver colored birds showed no coloration of the filtrate, indicating that an acid soluble diffuse pigment as described by Gortner (1911) was not present. Moreover, in the insoluble residue remaining after two hours of boiling in concentrated HCl, the same diffuse yellowish color was present wherever the black granules were clumped together as in the feather. Although an acid soluble pigment was found in red and buff feathers, its appearance in the acid filtrate has been asso-

ciated with the disappearance of the oval-shaped granules characteristic of these two types of feathers. Furthermore, in the insoluble residue of red feathers, this phenomenon also occurred wherever even a few of the round red granules are clumped together. It is improbable that a pigment diffused in the keratin could withstand two hours in boiling HCl, much less still fail to pass through ordinary filter paper. The absence of sufficient buff granules at any one locus immediately rules out the possibility that this phenomenon might be due to the presence of many granules which are out of focus, although this may accentuate the condition in some colors. Therefore, it would appear that only one tenable hypothesis remains for the explanation of this phenomenon in chicken feathers. Differences in size, degree of translucence and the color of the granules would cause them to have different optical properties. Therefore, the passage of light through a granule or the reflection of light from its surface would result in a diffusion of color throughout the adjacent keratin, the amount depending upon the density of the granules and the color dependent upon the color of the granules.

Willier and Rawles (1940) described the shape of the pigment granules as breed specific. Their study, however, considered only two types having definitely black pigmentation, the Black Minorca and the F_1 females from Barred Plymouth Rock females by Rhode Island Red males. They did not describe the differences found between the granules from these specimens. Hamilton (1940) was more explicit. He described the granules in cultured melanophores of Jersey Black Giants as "long, thin rods with truncate ends" in contrast to the short blunt rods of the Black Silkie bantam. Unfortunately no Jersey Black Giant feathers were available for checking this observation by the methods employed in this study. The term "breed" has also been used quite loosely by these workers. Hamilton includes the guinea with a list of poultry breeds. These workers, moreover,

described differences between the granules of red and black feathers and referred to these types as breed specific, even though varieties exhibiting either or both of these types of granules may be found within a single breed of poultry. On the other hand Lippincott (1921) found the rod-like granules of three black breeds to be identical. In the present study no apparent differences were found between the granules of any of the black types, even at a magnification of 1900 diameters. A similar condition also held true within all red, buff and blue varieties studied. It would appear, therefore, that the evidence favors a color or genotypic specificity of granule shape rather than a breed specificity.

CHEMICAL STUDIES OF FEATHER PIGMENTS
Materials and Methods

The feathers analyzed in this phase of the study were from the same samples as those utilized for the histological examinations of the previous section (Table 1). The observations on solubility were incidental to the preparation of acid residue for microscopic examination, and the preparation of basic solutions for spectrophotometric analysis. Therefore, all observations pertain to the solubility in hot and cold 6N HCl and 0.5N NaOH.

For the spectrophotometric analyses, 0.2 g. feather samples were weighed out on an analytical balance. For the sake of a uniform treatment, the feathers in the present study were all digested for two hours in 100 cc. of 0.5N NaOH and then allowed to cool. After standing for 48 hours, the samples were brought to volume and filtered through a fritted glass filter. Hydrolysis in all cases was nearly complete, since never more than a trace of pigment was left in the filter. According to Daniel (1938) the slope of the log. E curve for mouse hair was not affected by length of boiling time although a very short boiling time resulted in only partial hydrolysis. The only effect of varying the boiling period was to shift the line up or down on the graph as a result of variations in concentration.

The optical density of each solution was then determined by means of a visual spectrophotometer at wave lengths of 500, 600, and 700 millimicrons, with the average of 10 readings at each position establishing the data points. The reading from the instrument ($\log \frac{I_0}{I}$) is referred to as the optical density. In order to place the samples on a quantitatively comparable basis the optical density was converted to a 1 per cent. concentration and a 1 mm depth of solution, the resulting figure being the extinction coefficient ($E \frac{1\%}{1\,\text{mm}}$). For graphical presentation, the $\log E \frac{1\%}{1\,\text{mm}}$ values were plotted against wave length, since this produced a straight line for the pigments studied, while plotting the E values resulted in a curve. If two pigments studied are of the same chemical and physical composition, their spectral curves should coincide when equalized for concentration by simple vertical displacement. Variation in the slope of the curves, however, indicates qualitative differences between the samples. A numerical expression of the curves was obtained by taking the difference between the $\log E \frac{1\%}{1\,\text{mm}}$ values at 500 and 600 millimicrons, the resulting figure being referred to as the slope × 100. Only these two readings (500 and 600 millimicrons) were utilized in arriving at this value because they are less subject to error than is the point at 700 millimicrons due to the very weak absorption of the solutions at the latter wave length.

Solubility of the Pigments in Acid and Alkali

The solubility of the feather pigments of different breeds of poultry are indicated in Table 1. The pigments of all the self black, black and white, and blue feathers were resistant to the action of 6N HCl, no traces of pigment being found in the filtrate after hydrolyzing for two hours. Surprisingly, the pigment of Brown Leg-

horn cushion feathers seemed to be as resistant to the acid as was that from black feathers, even though their appearance indicated the presence of a light-colored pigment.

The pigment of Gold Campine and Partridge Plymouth Rock feathers showing a small amount of buff and red respectively alternating with black bars, was slightly soluble in the acid. The residue of the former showed rod-shaped granules characteristic of black feathers, while that of the latter showed rod-shaped and also a few round granules. Pigment from the red feathers showed a greater solubility in acid than pigment from the black, the Gold Campine, or the Partridge Plymouth Rock feathers due to the presence of a greater amount of of soluble pigment. However, round granules in the residue, which were not dissolved by long boiling, were characteristic of all red feathers. In regions of the plumage where black color appeared in the feathers, rod-shaped granules also appeared in the residue. Buff feather pigments were entirely dissolved by the acid in a very short time. A few acid insoluble pigment granules of varying shapes were found in all of the white breeds, which fact is in agreement with the histological observations already presented.

The presence of oval-shaped granules in red feathers similar to those in buff feathers (Table 1), along with the loss of the oval granules from both colors on acid hydrolysis, and the retention of round granules by the red feather residue, seem to indicate that only one pigment, buff, is soluble in concentrated hydrochloric acid. If this be true, it would strongly suggest that the red feather is genetically the same as buff with an acid insoluble brown pigment superimposed upon it. Each has been found from genetical analysis to carry the sex-linked gold factor.

All the pigments were completely dissolved by 0.5N NaOH but the rate of hydrolysis varied markedly. The acid insoluble pigment of the black appearing feathers

was only slowly soluble in the alkali, requiring about 45 minutes to an hour of boiling before being noticeably dissolved. Solubility was determined by examination of the filtered solution. Buff pigment on the other hand was apparently completely dissolved by the time the boiling point was reached, or about 10 to 15 minutes. Red feather pigments appeared to be the most soluble since their pigment began diffusing through cold alkali immediately upon introduction into it.

Differences in the alkali solubility between the black and the red or yellow pigments of various animals have been reported by other workers. Spöttel (1914) and Lloyd-Jones (1915) found such a difference in pigeons, Einsele (1937) in mice, Russell (1939) in guinea pigs, and Ladebeck (1921) and Görnitz (1923) in chickens. Görnitz also found that the red feathers contained a slightly more alkali soluble pigment than did the buff, and that both of these pigments were more readily soluble than the black pigments. Since the solubility of black pigments is expedited by oxidation with hydrogen peroxide, Görnitz postulated that the naturally occurring red and yellow bird feather pigments were oxidation products of black pigment.

Spectrophotometric Analysis

The slopes of the log. E curves for all feather solutions analyzed are given in Table 1. The slopes for all of the feathers showing only black pigment are the same, averaging – 0.33 with an extreme range of only 0.04. The curves for the blue feathers appear to be the same as those for black, the slope of – 0.34 and range of 0.07 being very close to the values for black pigment.

Buff feather pigment curves are distinctly different from the blacks, having an average slope of – 0.52 with a range of 0.22. The buff solutions were very dilute and were therefore difficult to read precisely, which may account for some of the variability of these samples. On the other hand, buff breeds frequently show small amounts

of black in the plumage which in such dilute solutions as these would have an appreciable effect on the slope of the curves.

The average slope of the curves for feathers from red breeds is – 0.63 with a range of 0.04.   This slope is decidedly more steep than that of buff or black feathers, indicating that this color is chemically distinct from either of the others.

The flattest curves were obtained from feathers of white breeds and had an average slope of – 0.28.   Due to the small amount of light absorbed by these samples, considerable relative variability would be introduced by traces of black pigment.   That this was the case is suggested by the fact that the range of the slope for the log. E curves was only 0.09 which is less than that of the buff samples.

The differences in slope exhibited by the solutions of different colored feathers appear to be very consistent and decided.   Zwicky and Almasy (1935), however, obtained absorption curves for black and red horse hair which were as widely different as were those from black and red feathers but nevertheless concluded that the two colors were not different entities.   In order to demonstrate that the variations in these curves are indicative of chemical differences rather than chance, statistical analysis was applied to a group of data.   For this statistical treatment three similar samples of Blue Andalusian feathers and five replicates from a representative breed of each of the four other self colors were used.   The samples were first washed in ether in an effort to further reduce the variability, and to determine whether any impurities so removed had affected the shape of the curves.   Such treatment, however, did not appreciably affect either factor.   The slopes of the absorption curves (Table 2) were then determined as previously described. The curves obtained are shown graphically in Figure 19. The analysis of variance for these slopes (Table 4) yielded an F value of 159.   Since the required value for

the 0.1 per cent. level is only 7.467, the probability of the five average slopes being from the same population is far less than one in a thousand. Further analysis
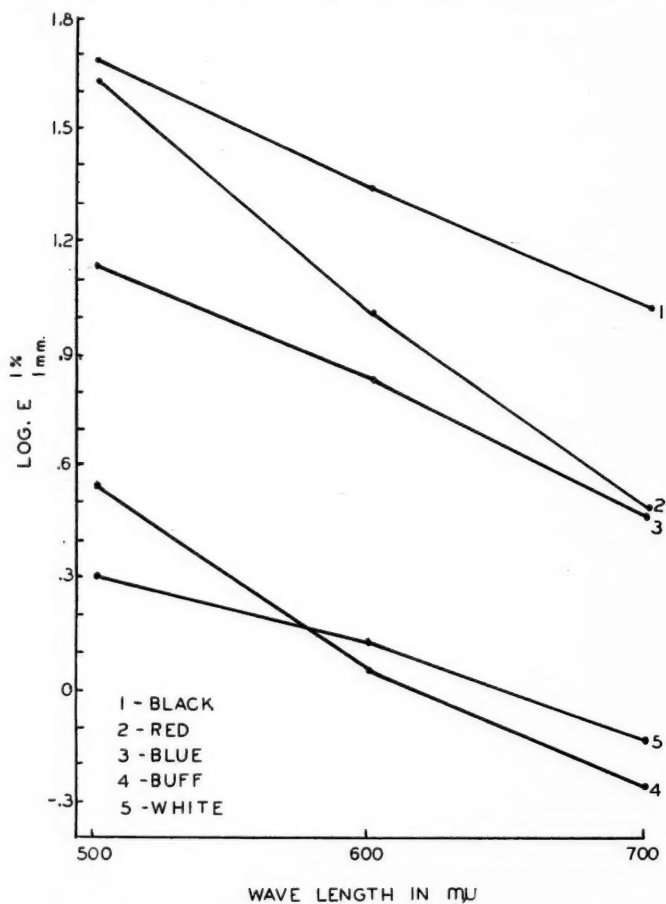
Fig. 19. Typical log. E curves for alkali solutions of various colored chicken feathers.

showed the least significant difference between any two averages at the 0.1 per cent. level of probability to be 0.0745. Since the difference between any pair of color

averages, except that between blue and black, is far greater than this figure, it is evident that the differences are statistically highly significant.   The actual difference between the average slope of blue and black feather pigments is 0.003 well within the 50 per cent. level of probability, 0.0138, indicating that the two sampling values are probably from the same population.

Baker (1942) was able by similar treatment to demonstrate an equally great difference between black and red guinea pig hair pigments.   However, Dunn and Einsele (1938) using the colorimeter and Daniel (1938) using the spectrophotometer to compare the absorptive power of various mouse pigments, concluded that the samples with which they worked varied only quantitatively.   Their evidence would appear quite conclusive until it is noted that these workers used only genotypes of the C series or Agouti series and that no true reds and yellows produced by the genotype ee were examined.   Gremmel (1939) also concluded that the black and red pigments of horse hair did not differ qualitatively.   Examination of Gremmel's data, however, show that the colorimetric comparison of the different colored samples gave exactly the results to be expected if red pigment is qualitatively different from black pigment.   It seems probable then, in spite of the conclusions of Zwicky and Almasy (1935), Daniel (1938), and others, to the contrary, that qualitative differences in the pigment of epidermal structures of animals do exist.

It is seen in Table 1 that the slope of the absorption curves of feathers containing both red and black pigment such as Gold Campine and Speckled Sussex are variable. The curves for the Gold Campine, Brown Leghorn, and Partridge Rock do not appear to differ from those of black feathers.   The absence of an acid soluble pigment in the Brown Leghorn feathers may explain their close resemblance to black, but the same explanation would not hold for the other two since they both contain an acid soluble pigment.   The similarity of the absorption curves

for feathers of these two breeds to those of black feathers may, however, be due to the proportion of the acid soluble pigment present being too small to affect the slope of the curve. When a larger proportion of the more soluble pigment is present as in Speckled Sussex body feathers and Rhode Island Red main tail feathers, the slope of the

TABLE 2

Comparison of the Slope (× 100) of the Log. E Curves of Different Colored Feathers.   (Ether Washed Samples)

Breed	Sample No.	Slope at 2 days (× 100)	4. 5 months		7 months	
			Slope (× 100)	Change from 2 days	Slope (× 100)	Change from 2 days
Black Minorca	B. 1	− 0.3170	− 0.4660	− 0.1490	− 0.4389	− 0.1219
	B. 2	− .2801	− .4484	− .1683	− .4425	− .1624
	B. 3	− .3233	.....	.....		
	B. 4	− .3028	− .4724	− .1696	− .4559	− .1531
	B. 5	− .3170	− .4895	− .1725	− .4522	− .1352
Average		− 0.3080*		− 0.1649		− 0.1431
Blue Andalusian	Bl. 1	− 0.3089	.....	.....	.....	.....
	Bl. 2	− .3036				
	Bl. 3	− .3045	− 0.4855	− 0.1810	− 0.4510	− 0.1465
Average		− 0.3057*		− 0.1810		− 0.1465
Rhode Island Red	R. 1	− 0.6289	− 0.6990	− 0.0701	− 0.6326	− 0.0037
	R. 2	− .6635	− .7235	− .0600	− .7825	− .1190
	R. 3	− .6498	.....	.....		
	R. 4	− .5935	− .8041	− .2106	− .8239	− .2304
	R. 5	− .5800	− .7570	− .1770	− .5901	− .0101
Average		− 0.6214*		− 0.1294		− 0.0908
Buff Orpington	Bu. 1	− 0.4274	− 0.6234	− 0.1960	− 0.4802	− 0.0528
	Bu. 2	− .5171	− .6716	− .1545	− .9508	− .4337
	Bu. 3	− .4461	− .6757	− .2296	− .5834	− .1373
	Bu. 4	− .4711	− .8013	− .3292	− .6460	− .1749
	Bu. 5	− .5544	.....	.....	.....	.....
Average		− 0.4922*		− 0.2273		− 0.1997
White Wyandotte	W. 1	− 0.2292	− 0.4776	− 0.2484	− 0.7213	− 0.4921
	W. 2	− .2240	.....	.....	− .6364	− .4973
	W. 3	− .1391	.....	.....	− .4191	− .2101
	W. 4	− .2090	− .7570	− .5480	.....	.....
	W. 5	− .1624	.....	.....	.....	.....
Average		− 0.1927*		− 0.3982		− 0.3998

* Least significant difference between any two means at 50% level = 0.015; at 0.1% level = 0.0863.

curves falls somewhat intermediate to those of black and red, closely approximating that of buff.

According to Beer's Law, if two different pigment solutions are combined one would expect the absorption of light at any given wave length expressed as E to be cumulative, and the curve for the mixed solution should fall between the curves for the pure solutions, the exact location depending upon the proportions in the mixture.

Therefore, proportional mixtures of red and black feath-
ers were prepared and their absorption curves deter-
mined (Table 3).   The purpose of this test was to deter-
mine whether it is possible to estimate accurately the
proportion of red and black in unknown solutions.   It is
evident from Figure 20 that the curves fall in order of
their proportion of the two pigments, and in Table 3 it
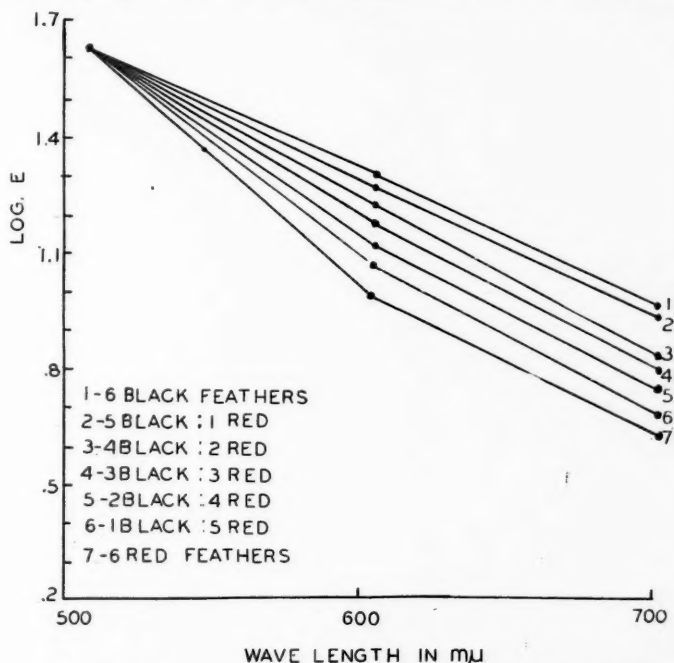
FIG. 20.   Log. E curves for mixtures of alkali solutions of red and black
chicken feathers, adjusted so that the values at 500 mμ coincide.

is shown that the curves of the mixtures fall almost pro-
portionally (on the E scale) between those of the pure
red and black feathers.

Zwicky and Almasy (1935) found that horse-hair solu-
tions were not altered on long standing.   However, Baker
(1942) found that the slope of the absorption curves for
solutions of black guinea pig hair changed from − 0.22

TABLE 3

SLOPE OF SPECTROPHOTOMETER CURVES FROM 500–600 MILLIMICRONS FOR MIXTURES OF RED AND BLACK FEATHER SOLUTIONS

Proportion	Experimental Slope	Expected Slope
Red	− 0.629	
5R : 1B	− .545	− 0.559
4R : 2B	− .503	− .499
3R : 3B	− .443	− .445
2R : 4B	− .397	− .398
1R : 5B	− .348	− .356
Black	− .317	

to − 0.48 in a period of four months. Since this change in slope is in the direction of red, he advanced this fact as evidence that red pigment is an oxidized form of black. Table 2 shows the effect of standing for four and a half and seven months on the slope of the absorption curve of chicken feather pigments. It is immediately clear that at four and a half months a change has occurred, although in no instance, except in the case of buff, was the change of as great a magnitude as the 0.26 shift reported by Baker. However, it is also seen that white feathers, which contain very little or no pigment, but only keratin, have undergone an even greater change than any of the chicken pigments or the black guinea pig pigment. It is therefore obviously fallacious to reason that a steepening of the slope of the absorption curves for colored feathers or hair is indicative of a change in the nature of the pigment when pigmentless feathers show a greater change. Baker did not report the effect of standing on white hair solutions. This point of criticism derived from white feathers, however, can not hold against the results of Arnow (1938) and Baker (1942) when they compared artificial red melanin, prepared by oxidizing

TABLE 4

ANALYSIS OF VARIANCE OF DIFFERENT FEATHER PIGMENTS

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	22	0.5900	
Between color means	4	.5735	0.1434**
Within groups (error)	18	0.0165	0.0009

** Value of F = 159; value required for significance at the 0.1 per cent. level = 7.467.

black dopa melanin which should be relatively free of impurities, with natural red pigment. The absorption curves of the two red melanins were identical, indicating that it would be possible for red melanin to be an oxidation product of black.

An equilibrium seems to have been reached by the white feather solution at four and a half months, and at seven months it had not changed further. On the other hand the colored solutions at seven months had all shifted back slightly toward the original value.

<div align="center">Summary and Conclusions</div>

Cushion feathers of 37 different breeds and varieties of poultry, consisting of reds, blues, blacks, buffs, whites and various gold and silver patterns were studied histologically and chemically.

<div align="center">*Histological Studies*</div>

Black feathers or feather parts showed rod-shaped granules of only slight variability in size averaging about $0.5\,\mu$ by $1.3\,\mu$. The granules were insoluble in 6N HCl, but slowly soluble in 0.5N NaOH.

Feathers of blue varieties contained very uniform round granules about $0.5\,\mu$ in diameter, distinctly different in appearance from those of red feathers. These were acid insoluble but were slowly soluble in the alkali.

Both dominant and recessive white feathers showed a few black pigment granules of irregular size in both web and fluff barbules.

Buff feathers contained oval-shaped granules averaging about $0.7\,\mu$ in diameter by $1.0\,\mu$ in length. These granules were readily soluble in either HCl or NaOH.

Red feathers contained oval granules similar in size, shape, and solubility to those in buff feathers. Round granules of very uniform size about $0.5\,\mu$ in diameter, very soluble in 0.5N NaOH and insoluble in 6N HCl were also found.

In all specimens except those of blue varieties, the distal (hooked) barbules were more heavily pigmented than the proximal (curved) barbules.

In all cases where round granules were found, they tended to be deposited in bead-like rows longitudinally oriented in the feather structure. Rod- or oval-shaped granules were also oriented longitudinally in the feather, but were not in such compact rows as were the red granules. In a short region of the barbule, adjacent to the barb of the feather, the precise organization of the pigment granules was lacking.

In general the web barbules were most heavily pigmented near the base and only there if the amount of pigment present was small. As the amount increased, the pigmentation extended more distally, the barbicels being the last structures pigmented. In black feathers pigment seldom extended to the tips of the barbicels.

The basal portions of the fluff barbs were also usually the most heavily pigmented. Here a definite distribution pattern within the internodes was also observed. The first place to be pigmented was in the distal part of the internode. As the pigmentation increased the granules extended more proximally within the internodes. The nodes were usually the last areas to be pigmented.

A hypothesis is presented to explain the condition which some workers have called diffuse pigment. Differences in size, degree of translucence and the color of the granules would cause them to have different optical properties. Therefore, the passage of light through the granule or the reflection of light from its surface would result in a diffusion of color throughout the adjacent keratin, the amount depending upon the density of the granules, and the color upon the color of the granules.

The granule shape of different pigments seems to be color specific rather than breed specific as suggested by some workers.

Chemical Studies

The slopes of the log. E curves for feathers of 32 breeds and varieties of poultry are given.

Analysis of variance for the average slopes of the log. E curves of black, red and buff feather pigments showed these pigments to be distinctly different one from another, and is contradictory to the conclusions of some workers that these colors differ. only quantitatively. Blue feather pigment is the same as that found in black feathers, and white feathers gave a significantly flatter curve than any of the color-bearing feathers.

Mixtures with known ratios of red and black feather solutions gave curves falling proportionally between those of pure black and pure red feathers. Thus a method is available for determining the relative amounts of the two pigments in unknown samples.

The steepening of the slope of the log. E curves, as affected by aging of the solutions, was demonstrated to be due largely to the change occurring in the keratin impurity since supposedly pigmentless white feathers exhibited changes of equal or greater magnitude. Such changes in colored feather or hair solutions, therefore, can not be interpreted as evidence of changes in the nature of the pigment.

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STATISTICAL ANALYSIS OF FACTORS
WHICH MAKE FOR SUCCESS
IN INITIAL ENCOUNTERS
BETWEEN HENS[1]

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THE basic importance of initial encounters, among certain animals at least, in many different major groups of phenomena (Collias, 1943) makes it seem important to analyze the factors deciding the outcome of such encounters. To make such an analysis I have taken advantage of the remarkable fact that the first thing two strange hens will do upon meeting is to settle their future dominance relations either by fighting or by passive submission of one bird. Once established the social order is very stable and is easily ascertained at any time by noting which hens peck which without being pecked in return. The social order has been observed to persist a year or more with no change in the pecking order.

METHODS AND RESULTS OF ANALYSIS

Two hundred pair contacts were staged in a neutral pen using normal moderately inbred white leghorn hens from different flocks. These contests were conducted over a period of three years, and their original purpose was merely to serve as controls in experiments designed to test the influence of various injected hormones on the out-

come of initial pair contacts. The care and housing of
the birds and the method of conducting the encounters
have been described in a previous publication (Allee,
Collias and Lutherman, 1939). Briefly, two hens from
different flocks were caught, weighed, state of moult was
noted, position in the social order in the home flock was
recorded, and standard comb measurements (length plus
height) were taken. The birds were then placed simul-
taneously in a strange pen purposely reduced to half size
to insure closer association. The time of the latent
period before contact and the length of the fight, if any,
were recorded along with notes on general behavior of
the birds toward each other. The statistical methods
used in analyzing the results are described below.

By artificially increasing certain variables, it is often
possible to discover which are real factors in deciding the
outcome of initial encounters. This makes it possible to
control those factors which can be readily controlled, and
by the application of statistical analysis to evaluate the
others and so to reach some idea of the relative impor-
tance of the different factors in a fairly normal situation.
It is the extent to which prediction can be attained that
is important, and the finer the discrimination possible,
the more nearly will rules apply to the vast majority of
individuals which cluster about the modes of species
variability rather than merely applying to the less numer-
ous variants at the extremes. The present report de-
scribes the progress attained toward this objective as
related to success in initial encounters.

A. Nature of the Encounters

In a typical fight the birds first become oriented with
respect to one another; as they examine each other the
face becomes red and flushed, the neck hackles rise, the
tail becomes more erect and the wings droop. The birds
may then jockey for a favorable position or at once leap
up and at each other and slash towards the head with the
beak in an attempt to seize and bite their opponent's comb

or wattles.   During the heat of battle the birds pay absolutely no attention to a human observer.   The fight often lasts but a few seconds.   The loser retreats and seeks to escape, its face pales, its feathers are depressed, it appears to be confused and panic stricken and looks for a place to hide, especially if closely and viciously pursued. The winner maintains much the same attitude as during the fight and only gradually does its excitement subside. Inhibition of the attack is based on experience, as well as subordination, since aggressive cocks or hens as a rule attack any strange individual, whereas attacks on flock mates are generally confined to relatively mild pecks. Fighting among hens, while often very strenuous, is much less severe than among cocks, and in the present series of encounters dominance relations were more often decided by passive submission of one bird than by an active battle.

B. *Controlled Factors*

1. *Sex.*   As a rule cocks dominate hens when full grown (Schjelderup-Ebbe, 1935).   Only hens were used in these experiments.

2. *Territorial defense.*   Of 1,428 first meetings of domestic fowl observed over a period of 10 years Schjelderup-Ebbe (1922) noted that the home bird "wished" to fight in 93 per cent. while the strange bird "wished" to fight in only 32 per cent., and the home birds won 62 per cent. of the 476 battles observed.   The courage of the new birds seemed lessened by their surroundings and they fought with less vigor.   All the encounters reported in the present study were conducted in a neutral pen which was quite similar to a part of the home pens of the birds concerned.

3. *Social facilitation.*   There is evidence that the presence of a powerful familiar despot betters the chances of its subordinates in contacts with strange individuals (Dr. A. M. Guhl, personal communication).   Since in the present experiment never more than two birds were intro-

duced into the fighting pen at the same time, this factor does not complicate the results.

C. *Statistical Analysis of Uncontrolled Factors*

These factors included the following readily measured features: (1) size of comb as an indicator of male hormone output; (2) weight as a partial indicator of strength, relative size, including "impressiveness" and general physiological state; (3) social rank in the home flock as a presumed indicator of the "psychology of success"; and (4) state of moult.

Previous experience had demonstrated that very unaggressive hens could readily be caused to win all their encounters with normal hens and to rise in the social order by injecting them with testosterone propionate (Allee, Collias and Lutherman, 1939). Possibly size of comb has some "impressive" or bluff value (*ibid.*). However, Dr. A. M. Guhl (unpubl.) in this laboratory recently found that treatment of dubbed hens with testosterone caused such hens to rise in the social hierarchy; local application of this androgen induced considerable increase in comb size of normal hens, but had no influence on social rank. It had also been found that doses of thyroxin large enough to cause moulting and a marked reduction in body weight as well as to inhibit the gonads would considerably reduce the chances of a hen of winning its initial encounters (Allee, Collias and Beeman, 1940).

Size of comb was recorded in millimeters, weight in ounces, moult by arbitrary grades and social rank in terms of numbers of subordinates. For purposes of statistical analysis moult of the winner was simply treated as greater or less than the moult of the loser, while rank was adjusted for difference in size of flock by a transformed scale making use of inverse probability functions. For the latter purpose use was made of the following formula given me by Professor Wright.[2]

[2] Prf $\dfrac{x}{\sigma}$ is the area of the unit ($\sigma = 1$) normal curve between the mean (0) and x/σ and prf^{-1} is the symbol for the inverse function and gives the devia-

$$\text{Adjusted rank} = \text{prf}^{-1}\left(\frac{\text{Number pecked} + \frac{1}{2}}{N} - \frac{1}{2}\right)$$

We are assuming that the bird is located at a point which dichotomizes a normal distribution in such a way that the subordinates are on one side and the dominants are on the other. The bird itself is assumed to contribute equally to both sides. The proportion of subordinates is thus taken as $\dfrac{\text{number pecked} + .50}{\text{size of flock}}$. The proportion of subordinates between the mean of the hypothetical scale and the rank of the given bird is then $\dfrac{\text{number pecked} + .50}{\text{size of flock}} - \dfrac{1}{2} = \text{prf } R$. The inverse function ($\text{prf}^{-1} R$) gives us the value of the adjusted rank on the hypothetical scale. The following diagrams may help to make this clear.
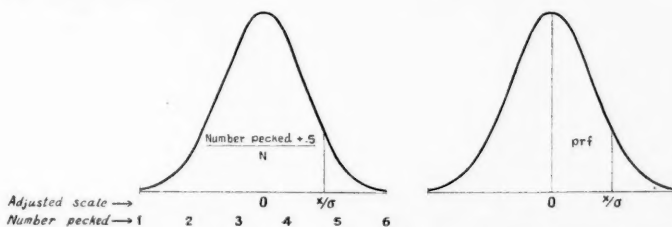
FIG. 1. Diagrams to illustrate statistical adjustment of rank in peck order to size of the home flock.

In a flock of 7 birds (maximum of 6 subordinates for highest bird) the adjusted rank for a bird which pecked 5 subordinates would be $\text{prf}^{-1}\left(\dfrac{5 + .50}{7} - .50\right) = .79$. This formula tends to bunch together the birds near the center of the social scale on the assumption that the underlying factors which make for success would exhibit a normal distribution if a large enough population were considered. All the statistical methods used in this report were based on the same assumption. All modifications of the usual

tion (in terms of σ) that underlies the designated area. Tables of these functions are provided by Davenport and Ekas (1936).

statistical methods which are used were kindly provided
to me by Professor Wright.

The first step was to correlate each factor with success.
The two hundred fights, rather than the individual birds,
have been taken as the units throughout in obtaining cor-
relation coefficients. A preliminary tabulation indicated
that even in contests conducted between the same pair of
individuals at different times the results were frequently
reversed. It must be remembered that these hens came
from different flocks and did not associate with each other
between these staged fights. If successive pair contacts
are well spaced in time, each becomes essentially an initial
contact. Hence such variability is not incompatible with
the relative permanence of social order in stable flocks of
hens. None of the data were grouped.

The variables that have been considered are the differ-
ences between the birds involved in the fight, not the
absolute measurements. Each fight may be considered
from the view-point of either bird as the one whose victory
or defeat is to be used in describing the results and from
whose grades in the various characters, those of the other
bird are to be subtracted to obtain the differences. Thus
a fight in which bird A, weighing 3 lbs. 8 oz., defeated
bird B, weighing 3 lbs., may be entered in the correlation
table relating success and difference in weight either as a
''victory'' associated with a difference of + 8 oz. or a
''defeat'' associated with a difference of − 8 oz. As this
is an arbitrary matter, it is legitimate to make entries
from both viewpoints, thereby making the marginal fre-
quencies of the correlation table symmetrical (as in the
case of a fraternal correlation table). In the correlations
involving success, the complete table would have 200
entries in the category ''victory'' and 200 entries in the
category ''defeat.'' The total distributions of the differ-
ences in weight, comb size, rank and state of moult are
necessarily symmetrical about zero.

In determining the signs of the entries, it is assumed
that the winner of a fight stood higher than the loser on a

hypothetical scale describing the array of factors making for success. Thus "victory" is treated as positive, "defeat" as negative. A difference in rank is treated as positive if the first bird (*i.e.*, the one whose grade is to be treated as minuend) stood higher in the peck order of its flock than the second, according to the scale discussed above. With regard to the state of the plumage, it has seemed best to treat freedom from moult as highest in the scale and extreme moult as lowest. In this way, the differences which one might expect to find associated with success (*viz.*, those that go with greater weight, greater comb size, higher rank and relative freedom from moult) should exhibit positive correlations with success if, as turns out to be the case, this expectation is realized.

The correlations between success and the differences in the graded characters, weight, comb size and rank, were obtained by Pearson's coefficient of biserial correlation (in the form given by Kelley, 1923).

Let S be the difference between a first and a second bird on a hypothetical scale based on the factors making for success in fighting. A positive value implies that the first bird won, a negative value that it lost. It is assumed in the theory that the differences are distributed normally on this scale.

Let W be the weight of the first bird minus that of the second.
Let W_1 be a value of W in a case in which the first bird won.
Let W_2 be a value of W in a case in which the first bird lost.
$\overline{W}_1 = \Sigma W_1/200$, $\overline{W}_2 = \Sigma W_2/200$ are the means.
If each fight is entered in both ways $\overline{W}_1 = -\overline{W}_2$, $\overline{W} = 0$.
Thus $\sigma_W = \sqrt{\Sigma W^2/400} = \sqrt{(\Sigma W_1^2 + \Sigma W_2^2)/400} = \sqrt{\Sigma W_1^2/200}$ is the standard deviation of all differences in weight about zero.

The formula for biserial correlation is as follows:

$$r_{SW} = \left(\frac{\overline{W}_1 - \overline{W}_2}{\sigma_W}\right)\left(\frac{pq}{z}\right)$$

where p and q are the proportions in the two categories with respect to success and z is the ordinate of the unit normal curve at the point of dichotomy. In the present case $p = q = 1/2$, $z = 1/\sqrt{2\pi} = .399$. $r_{SW} = 1.253\ \overline{W}_1/\sigma_W$.

Differences in comb size (C) and rank (R) were treated similarly.

The standard errors of these correlations were obtained from Soper's approximate formula as given by Kelley (1923).

$$SE_r = \frac{1}{\sqrt{N}} \left(\frac{\sqrt{pq}}{z} - r^2 \right) = (1.253 - r^2)/\sqrt{200}.$$

The case of moult (differences represented by M) requires further consideration. Three categories were recognized: more, equal and less moult. It is assumed that these represent a trichotomy of a normal distribution of a scale of graded differences. Let a, b and c be the proportions with more, equal and less moult respectively in any distribution. Assume that the threshold between more and equal moult is at $-.50$ on this scale and that that between equal and less moult is at $+.50$. The difference between these thresholds can be expressed as follows in terms of the standard deviation (*cf.* Wright, 1934a).

$$[prf^{-1}(a + b - .50) - prf^{-1}(a - .50)]\sigma = 1$$

If a frequency between mean and threshold comes out negative as calculated from $(a + b - .50)$ or $(a - .50)$ the sign of the inverse probability function is to be taken as negative.

Among the winners there were 16% with more moult than the loser, 32% with equal moult and 52% with less moult. This yields for the standard deviation of this category (σ_{M_1}).

$$\sigma_{M_1} = 1/[-prf^{-1}(.02) + prf^{-1}(.34)] = 1.059$$

The location of the mean of the winners on our hypothetical scale can now be obtained.

$$\overline{M}_1 = .50 - \sigma_{M_1}prf^{-1}[a + b - .50] = .50 + 1.059 \; prf^{-1}(.02) = .553$$

Because of symmetry, the mean of the losers ($\overline{M}_2$) is $-.553$ on this scale.

The variance of the total (σ_M^2) is compounded of the average within the rows (both $1.059^2 = 1.122$) and that between them (which is $.553^2 = .306$). Thus $\sigma_M^2 = 1.122 + .306 = 1.428$, $\sigma_M = 1.195$.

The standard deviation of the total could also be calculated directly from the symmetrical trichotomy of the total ($a = .34$, $b = .32$, $c = .34$).

$$\sigma_M = 1/[prf^{-1} \; .16 + prf^{-1} \; .16] = 1.212$$

The discrepancy between these estimates is due to the fact that if the distributions of the separate rows are normal (as assumed in calculating their means) the distribution of the total is more or less platykurtic, instead of normal (as assumed in the second estimate of σ_M). Properly it is this total (based on birds taken in both orders with respect to success and therefore equivalent to ones taken in random order) that should be considered as normal, but for consistency with the calculation of the row means, the estimate $\sigma_M = 1.195$ is used here. The error in assuming normality of the distributions within the separate rows does not appear to be important as indicated in some measure by the comparison of the two estimates of the total standard deviation.

The biserial correlation may now be estimated.

$$r_{SM} = 1.253 \ M_1/\sigma_M = .580 \pm .065$$

This has been assigned a standard error by the same formula as in the preceding cases, although this is undoubtedly somewhat of an underestimate because of the nature of the scale. There is, however, no doubt of the significance of this correlation.[3]

In order to estimate the relative importance of the different factors it was necessary to take into account their correlations with each other. These correlations were obtained by use of the ordinary product moment formula in the case of differences in weight, comb, and social rank.
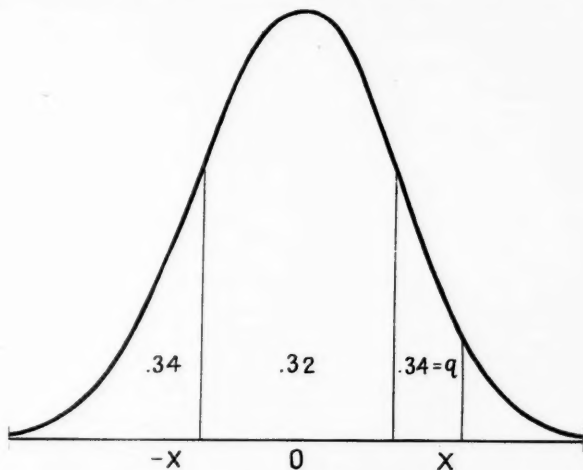
FIG. 2. Transformation of values for moult to a numerical scale on the normal probability curve.

Pearson's broad category method was used in the cases in which moult is involved. The marginal frequencies (34% more, 32% equal and 34% less moult) are here assumed to trichotomize a normal probability curve with unit standard deviation, a different scale from that used above. The mean of the middle category is obviously zero. The mean deviation (d) for the category "less moult" is given by the following formula in which q is

[3] Professor Wright, personal communication.

the tail frequency (.34) and z is the ordinate at the threshold.

$$d = z/q = .3664/.34 = 1.078$$

The deviation of the category "more moult" is obviously -1.078.

$$\sigma_d{}^2 = (.34d^2 + 0 + .34d^2) = .790$$

is the variance as estimated from the mean deviations. The true variance is 1.

According to Pearson's formula,

$$r_{MW} = \frac{\Sigma dW}{N\sigma_W\ \sigma_d{}^2}.$$

The formulae for r_{MC} and r_{MR} are similar.

All these correlations between factors have been assigned standard errors by the usual formula, $\sigma_r = (1 - r^2)/\sqrt{N}$ although this is undoubtedly slightly too low in the cases involving moult, because of the coarseness of the scale.

We assume that the array of factors upon which the result of a fight depends includes cases in which differences in weight, comb size, state of moult and social rank are close indicators. In Figure 3 the correlations are known and are indicated by curved lines. The object is to find the value of coefficients measuring the influence along the paths indicated by arrows. An estimate can be obtained by the method of path coefficients (Wright, 1921, 1934a, 1934b) which in a symmetrical system such as used here is essentially the same as that of multiple regression, except for qualifications from the hypothetical character of the scale in the case of the differences in state of moult, social rank and success. The correlation between success and one of the factors such as weight may be analyzed into a direct contribution measured by the coefficient for the path from weight to success and three indirect contributions measured by the products of the coefficients along the paths from W through C, M, and R respectively to S. The correlations between success and the four factors provide four simultaneous equations which are identical with the normal equations of multiple regression.

$$r_{SW} = p_{SW} + p_{SC}r_{WC} + p_{SM}r_{WM} + p_{SR}r_{WR}$$
$$r_{SC} = p_{SW}r_{WC} + p_{SC} + p_{SM}r_{CM} + p_{SR}r_{CR}$$
$$r_{SM} = p_{SW}r_{WM} + p_{SC}r_{CM} + p_{SM} + p_{SR}r_{MR}$$
$$r_{SR} = p_{SW}r_{WR} + p_{SC}r_{CR} + p_{SM}r_{MR} + p_{SR}$$

Table 1 summarizes the correlation coefficients and the path coefficients resulting from solutions of the normal equations.

TABLE 1

CORRELATIONS AND PATH COEFFICIENTS RELATING DIFFERENCES IN CHARACTERS TO SUCCESS IN FIGHTS FOLLOWING INITIAL ENCOUNTERS

Difference	Correlation with success	Path coefficient relating to success
slightness of moult	.580 ± .065	.417
comb size	.593 ± .064	.354
weight	.474 ± .073	.111
rank in own flock	.262 ± .084	.209

Multiple correlation = $\sqrt{\Sigma rp}$ = .748

TABLE 2

CROSS CORRELATIONS AMONG THE CHARACTER DIFFERENCES

Character differences	
Slightness of moult and comb	.379 ± .061
Slightness of moult and weight	.387 ± .060
Slightness of moult and rank	−.064 ± .070
Comb and weight	.440 ± .057
Comb and rank	.156 ± .069
Weight and rank	.220 ± .067

TABLE 3

DEGREE OF DETERMINATION OF SUCCESS BY INDICATED FACTORS

Moult	.174
Comb	.125
Weight	.012
Social rank	.044
Moult and comb, joint residual	.112
Moult and weight, joint residual	.036
Moult and rank, joint residual	−.011
Comb and weight, joint residual	.035
Comb and rank, joint residual	.023
Weight and rank, joint residual	.010
Total determination (= .748² =)	.560
Residual determination =	.440
	1.000

These results need some interpretation. The correlation coefficients measure the total influence direct and indirect, of the various factors. The multiple correlation coefficient (.748) indicates the correlation between the best linear function of all of the factors and success.

Because of inter-correlations, the correlation coefficients do not however give a reliable evaluation of direct effects of the factors. The relatively high correlations between moult, weight, and comb size may be an expres-
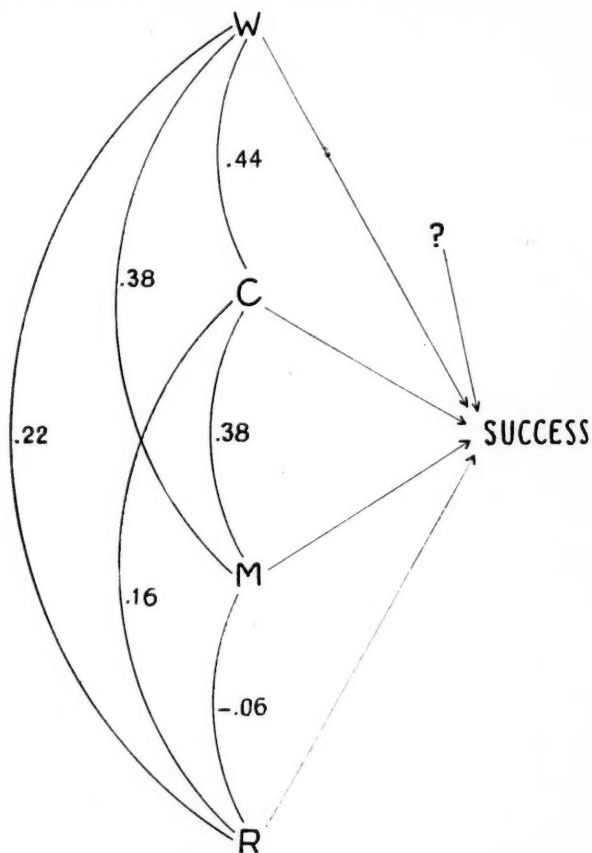
Fig. 3.   Interrelations of factors influencing result of initial encounters.

sion of pituitary action but they can be more directly explained as being due in large part to a common factor which is presumably the amount of thyroxin which is present since this hormone in fairly large doses will in-

duce moulting, reduce weight and suppress the gonads, all of which are more or less associated with loss of combat. The correlation between social rank and weight (.220) between social rank and comb size (.156) are low. The correlation that does exist may be a result of the fact that differences in weight and androgen which helped decide the original rank have persisted in part. Social rank once decided is much less variable than is weight, size of comb, or state of moult, hence the correlation is low. Then, too, birds high in the peck order have precedence to food and probably thrive better. The very low correlation between moult and social rank (−.064) is probably of no significance.

Inspection of the tables indicate that according to the path coefficients, moult (absence) was the most important factor deciding success, followed by male hormone indicated by comb, social rank and weight in that order. The small influence of weight is noteworthy. Moult exerts the greatest relative influence, but exactly how it does this is not known since it has been statistically freed from its actions through weight or the gonads. Perhaps it tends to make the bird more retiring since moult is really a kind of physiological illness, perhaps it makes the bird more sensitive to blows received, and perhaps the loss of feathers renders a bird less able to maintain equilibrium and to fight effectively. Size of comb is one of the best convenient indicators of male hormone output which we know at present. Hens secrete male as well as female hormone and injection of androgen has been shown to have a very marked and stimulating effect on the outcome of initial encounters unlike injection of estrogen (Allee, Collias and Lutherman, 1939; Allee and Collias, 1940). Social rank is perhaps a somewhat objectionable criterion since it may conceal factors of the very nature we are trying to uncover. However, it was the most convenient indicator of the "psychology of success" available, and furthermore this objection is obviated to a substantial degree by the low correlations of moult, comb and weight

with social rank as compared to their relatively high
correlations with success in initial encounters. The
rather small importance of social rank as an independent
factor as indicated by its path coefficient is also note-
worthy in this respect.

The degrees of determination of the variance by the
various factors reduce the results to a percentage basis
and give a good idea of how the different factors influence
variability in degree of success, acting singly and in com-
bination. Acting singly, they necessarily exhibit the
same order as their path coefficients since they are merely
the squares of the former. Joint determination repre-
sents the contribution to variability (plus or minus) due
to correlated occurrence of the two factors under con-
sideration. The order of joint determinations obviously
may differ from the order of the intercorrelations since
the latter do not measure degree of influence on success.
For example, moult and weight are more closely corre-
lated than moult and comb, yet the joint determination
by the latter pair is almost four times as great as that
by the former pair which involves weight, a relatively
ineffectual factor. One of the important properties of
the degree of determination is that the sum of the
components must be equal to 1. The general equation is
$\Sigma d_{x \cdot A} + d_{x \cdot \overline{AB}} = 1$, which is read, the sum of the deter-
mination of x due to A and the sum of the determination
of x due to the correlation between A and B must be equal
to 1 (Wright, 1921). This gives a means of estimating the
percentages of unknown factors (residual) by subtrac-
tion. In the present case 56.0 per cent. of the factors are
accounted for by the single or correlated action of moult,
comb size, weight and social rank, and presumably the
underlying factors of which they serve as more or less
imperfect indicators are responsible for a somewhat simi-
lar percentage. It follows that 44 per cent. of the fac-
tors were not measured in this analysis or were not
known.

D. Unmeasured and Unknown Factors

One possible way to account for the unknown factors lies in the probability that more accurate indicators of the underlying factors would have given higher values for the paths, cutting down the amount assigned to residual factors. This is not to assume that still other factors do not exist which are unrelated to these indicators.

There are certain known but unmeasured factors which influence success and may have complicated the results to an unknown extent in the present case. Among these are age, the behavior of the other bird, and previous fighting experience.

Young pullets up to at least 9 months of age are somewhat refractory to injected androgen in terms of the stimulation of aggressive behavior (Allee, Collias, and Lutherman, 1939). Some of the birds used were barely older than this and included flock-mates of the treated birds. When encounters involving birds less than one year of age with older birds were omitted, the multiple correlation coefficient fell slightly from 0.75 to 0.70. The path coefficients were almost identical except that that for the comb size fell to 0.31 from 0.35. Possibly difference in general size weakened the accuracy of the comb as an indicator since the older hens tended to be somewhat heavier and larger. On the other hand, senility may play a role. Degenerative changes occur in the pituitary of old fowl (Payne, 1941). There is some recent evidence which suggests the possibility that senile animals may become less sensitive to injected androgens (Hoskins, Levine and Bevin, 1939). One flock of our hens was approaching senility, but were still laying, during the last set of encounters in which they were used. However, no effect of age differences on success is evident from the gross data; of the 200 encounters, 36.5 per cent. were won by older birds, 39.5 per cent. by younger birds, and in 25 per cent, of the cases the opponents were of about the same age.

The behavior of the other bird is undoubtedly important. A hesitant individual will often take heart and attack when it observes that its opponent seems frightened. In only 33.5 per cent. of the encounters did an actual battle ensue, and 78 per cent. of the encounters were won by the bird that started the encounter. The longer the latent period before contact began the shorter a fight was likely to be and the greater the probability that one bird would submit passively.

Experience in winning or losing can be an important factor at times, and by purposeful application of this factor the results of a later encounter between a given pair of birds can often be reversed in a later encounter. It is a little difficult to extract suitable test cases in encounters of hens. Seven cases were tested in which the original loser had won from two to five encounters while the original winner had lost from three to six encounters just before the second meeting, and the results were reversed in every case. Ginsburg and Allee (1942) have recently brought out the marked importance of this factor of conditioning in mice by a series of carefully controlled experiments. Whether conditioning played much of a role in the present series of encounters is somewhat debatable, but a conscious effort was made to control this factor by not fighting any bird more than once a day. The more immediate history of a bird might perhaps be expected to exert the greatest influence; however, it is interesting to note in this connection that the correlation between winning a given encounter and success in the preceding encounter was exactly the same as the correlation with the second preceding encounter, that is, 0.66. This tetrachoric correlation was conveniently secured by use of computing diagrams prepared by Thurstone and Chesire.

Endurance is probably a factor when an actual fight takes place. By fighting the same tough hen over and over again in succession Schjelderup-Ebbe (1922) was able to make her yield and be subordinate to weaker

birds. During a battle it is obvious from the increasing slowness of movements of the birds that they are becoming more and more tired. Since none of the hens were fought more than once a day in the present series of encounters some measure of control over this factor was afforded.

The nature and importance of more completely unknown factors can merely be speculated on at present— differences in fighting skill, chance blows, differences in sensitivity to hormones, wildness, mild indisposition akin to illness, resemblance of the opponent to a despot or subordinate in the home flock, the details of past history, minor external disturbances, slight differences in handling, and errors in measurement perhaps have an aggregate effect of significant dimensions.

APPLICATIONS OF THE METHOD AND RESULTS

The significance and general implications of the problem will be developed elsewhere (Collias, 1943). The general importance of the statistical method employed is that it provides something of a model whereby the relative importance of the various factors which decide social dominance relations in various situations of ecological significance may be quantitatively evaluated. The present statistical method of evaluating the factors which make for high social level would have to be modified for each particular case, but this would probably not entail insurmountable difficulties.

One of the biggest complications and not adequately dealt with in this report since its influence was experimentally eliminated, is the interrelationship of territory. Schjeldrup-Ebbe's data indicate the possibility that in chickens, which have not generally been regarded as territorial birds, this factor may often outweigh all the others. However, even in nature, birds may on rare occasions be displaced from their territories by stronger rivals. Perhaps territorial influence could be measured by the distance from the territorial center.

More specific parallels of the general results to natural situations, at least cases where one factor apparently dominates the situation, can be cited even in the present inadequate state of investigation of social hierarchies in the vertebrates. At first thought, the size and weight factor is perhaps most obvious, *e.g.*, a general order of precedence to food in rough order of size differences is very common, for example, in birds of different species at a feeding shelf (Nice, 1929), or in different species of ducks and geese on a park lagoon (Jenkins, in press). The factor of male hormone is probably the important one in many situations. Males usually dominate females, and Lorenz (1931) states that young jackdaws rise in the peck order as they become reproductively activated, and much the same thing has been found by Shoemaker for canaries (1939). Coveys of quail and winter flocks of many other birds break up with the onset of the breeding season and the parallel rise in gonad activity and individual aggressiveness. Fighting is very common in groups of young male mammals (Alverdes, 1935). Examples of the action of thyroxin are more obscure and lie on more unstable assumptions, because of the complexity of its effects. In general, birds when moulting tend to retire to dense cover and hence to avoid conflict with other members of their species. The same retiring habit is seen during incubation when gonad activity is likewise reduced. Schjelderup-Ebbe (1922, 1935) has described a decided increase in aggressiveness of broody hens and such accords with the popular idea, but some evidence has been gathered in this laboratory which, while inadequate in itself, suggests the need for caution in relating such things as the defense of her chicks by a broody hen to position in the social hierarchy of the flock. The effective stimuli for types of aggressive behavior with different physiological bases may be very different.

Summary

Initial encounters lie at the basis of the social order in flocks of chickens, as is known to be the case with a num-

ber of other vertebrates. To gain some insight into the factors which decide the outcome of initial encounters 200 pair contacts were staged in a neutral pen using normal, moderately inbred white leghorn hens from different flocks. Controlled factors included sex, territorial familiarity, and social facilitation. Evaluation of the less easily controlled factors was made mainly by the use of a modified biserial correlation formula. The degrees to which success in encounters was controlled by the more important factors were determined by the method of path coefficients which in the present instance was the same as the method of multiple regression.

Factors of major importance were male hormone output as indicated by comb size and thyroxin secretion as indicated by the complex of changes which accompany moulting. Social rank in the home flock had much less influence, and weight was of only small importance.

The multiple correlation coefficient of success with the four factors analyzed was 0.75. Forty-four per cent. of all factors were unknown or unmeasured in this analysis.

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PROOF FOR MULTIPLE ALLELISM OF SEX–DIFFERENTIATING FACTORS IN HABROBRACON[1]

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In the wasp Habrobracon, sex determination is complementary. Haploid males, similar in phenotype, are of different recessive genotypes. Diploid males, likewise similar in phenotype, are of different homozygous recessive genotypes, corresponding to those of the haploid males. Females are produced by any of the heterozygous combinations of the different male sex genotypes.

Snell (1935) proposed a multiple factor theory for sex determination in Habrobracon, according to which females are developed if the zygote is heterozygous for one or more independently segregating factor pairs, $xa/xb, ya/yb, za/zb$. Males must be homozygous (diploid) or azygous (haploid).

Whiting (1940) analyzed previously published data, showing them to be consistent with a multiple allele theory. According to this theory, the normal haploid males would have any one of a series of sex alleles (xa, xb, xc, xd, etc.), the corresponding diploid males would be homozygous (xa/xa, xb/xb, xc/xc, etc.), and the females heterozygous (xa/xb, xa/xc, xb/xc, etc.). With n alleles there would be n different kinds of haploid males, n corresponding homozygous diploid males and $\frac{n^2 - n}{2}$ heterozygous "double dominant" female-producing combinations.

No intersexes have ever been found associated with any of the sex differentiating genotypes of Habrobracon. The diploid males are in every way similar to the

haploid males, except for differences usually associated
with doubled chromosome number (cell size, etc.). They
show no evidence of feminization either in primary or in
secondary sex traits.

Fraternities containing diploid males have previously
been called closecross fraternities because in general the
parents have been closely related, while fraternities con-
taining no diploid males have been called outcross fra-
ternities since these are usually produced from unrelated
or distantly related parents. On the basis of the multiple
allele theory, these two types of fraternities may now be
more exactly designated two-allele and three-allele fra-
ternities.

Sex ratio in Habrobracon depends, among other things,
upon ratio of eggs fertilized. Under standard incubator
conditions this is roughly two thirds, but there is much
fluctuation. When conditions are otherwise uniform,
ratio of females to haploid males in three-allele fraterni-
ties is twice that in two-allele fraternities since all zygotes
are heterozygous (female-producing) in the former case,
but only half are heterozygous in the latter. The homo-
zygous (diploid male-producing) combinations constitut-
ing the remaining half of the zygotes in the two-allele
fraternities are of low viability, so that there are many
unhatchable eggs and ratio of diploid males to females is
very much lowered from unity. This ''diploid male
viability ratio'' measures the viability of the diploid
males which is very variable relative to that of the females
which is high and very stable.

Preliminary Experiment

Every F_2 male (haploid) from crosses of stocks with
different sex alleles, xa/xb and xc/xd, when mated to
females of both parental stocks, should fall into one or
the other of two classes, those (xc and xd males) siring
diploid sons with xc/xd females but not with xa/xb, and
those (xa and xb males) siring diploid sons with xa/xb
females but not with xc/xd.

No diploid males have ever been obtained from crosses between stocks 17-ivory and 36-veinless, indicating that these stocks have different sex alleles and they were therefore selected for use in the following experiment. The mutant genes ivory, o^i, and veinless, vl, served as markers to separate the haploid males from their diploid brothers.

Each of 17 F_2 males from reciprocal crosses when back-crossed sired diploid sons by stock 36-veinless females ($+ \female\female$ 1390, $vl \male\male$ 902, $+ \male\male$ 95) but failed to sire them by stock 17-ivory females ($+ \female\female$ 1644, $o^i \male\male$ 912). Ratio of females to haploid males in the latter cross, 1.803, is higher than that in the former, 1.541, indicating replacement of diploid males by females in the two-allele fraternities. In this case the low viability of the veinless males decreases the expected 2:1 proportion between the female ratios of the three-allele and two-allele fraternities to 1.170:1.

Each of 17 F_2 males when back-crossed sired diploid sons by stock 17-ivory females ($+ \female\female$ 1102, $o^i \male\male$ 1213, $+ \male\male$ 105) but failed to sire them by stock 36-veinless females ($+ \female\female$ 2193, $vl \male\male$ 796). Low viability of veinless exaggerates the difference in female to haploid male ratio in these crosses 3.03:1 instead of 2:1.

Two males sired no diploid sons by females of either parental stock. Among their offspring from veinless females the daughters were 3.27 times as numerous as the sons ($+ \female\female$ 108, $vl \male\male$ 33) and from ivory females the daughters were only 0.91 times as numerous ($+ \female\female$ 95, $o^i \male\male$ 104). Difference in ratio of daughters to haploid sons indicates that these two males had the same sex alleles as the ivory females and that the lack of diploid sons is an error of sampling.

Ratio of diploid males to females (diploid male viability ratio) is 0.090 for all the two-allele fraternities.

According to Snell's (1935) multiple factor hypothesis crosses of two stocks with the same sex-differentiating pair will produce only female-developing zygotes if the

stocks differ by one or more sex factors for which each is homozygous. With two differences involved, the stocks may be designated xa/xb ya/ya za/za and xa/xb yb/yb zb/zb. All zygotes from crosses of these two stocks will be female because ya/yb za/zb although half would be homozygous for x. If the x pair be disregarded because both members are present in each stock, the F_2 males will fall into four equal classes, $ya.za$ and $yb.zb$ which will sire diploid sons with one stock but not with the other and $ya.zb$ and $yb.za$ which will sire diploid sons with neither stock. Thus only half of the F_2 males can sire diploid sons in the tests. If the stocks differ by three factors only one fourth can sire diploid sons, if by four factors, only one eighth, etc. The data presented above preclude the possibility that two or more different pairs of sex factors characterize the stocks, if the sex-differentiating factors are the same.

If the sex-differentiating factors of the two stocks are different, xa/xb ya/ya za/za and xa/xa ya/yb zb/zb, certain F_2 males produced by some of the F_1 females can sire diploid sons by females of neither stock. Data presented above are not regarded as adequate entirely to preclude this possibility. These data are also consistent with the two factor hypothesis of xa/xb ya/ya and xa/xb yb/yb stocks according to which half of the F_2 males will sire diploid sons by xa/xb ya/ya females but not by xa/xb yb/yb females and half will show the reverse result. This is also as expected for quadruple alleles.

Use of the Recessive Sex-Linked Gene Fused in Testing Multiple Allelism of the Sex Factors

In two-allele fraternities sex-linkage of fused is obvious with about 10 per cent. crossing-over. If fused is associated with the same sex allele in the female as in the male, the majority of the daughters will be wild type, of the diploid sons fused.

P_1 $\qquad$ $\dfrac{xa}{xb\ fu}$ ♀ × $xb\ fu$ ♂

F_1	females	diploid males
non-crossovers	$9\ \dfrac{xa+}{xb\ fu}$	$9\ \dfrac{xb\ fu}{xb\ fu}$
crossovers	$1\ \dfrac{xa\ fu}{xb\ fu}$	$1\ \dfrac{xb+}{xb\ fu}$

If fused is associated with different sex allele in the parents, the majority of the daughters will be fused, of the diploid sons wild type.

P_1 $\qquad$ $\dfrac{xa+}{xb\ fu}$ ♀ × $xa\ fu$ ♂

F_1	females	diploid males
non-crossovers	$9\ \dfrac{xb\ fu}{xa\ fu}$	$9\ \dfrac{xa+}{xa\ fu}$
crossovers	$1\ \dfrac{xb+}{xa\ fu}$	$1\ \dfrac{xa\ fu}{xa\ fu}$

In three-allele fraternities sex-linkage is masked since all zygous offspring are female.

P_1 $\qquad$ $\dfrac{xa+}{xb\ fu}$ ♀ × $xc\ fu$ ♂

F_1	females	females
non-crossovers	$9\ \dfrac{xa+}{xc\ fu}$	$9\ \dfrac{xb\ fu}{xc\ fu}$
crossovers	$1\ \dfrac{xa\ fu}{xc\ fu}$	$1\ \dfrac{xb+}{xc\ fu}$

Bostian (1939) by a prolonged inbreeding experiment discovered the presence in his stock of triple alleles governing sex. Females heterozygous for fused were in each generation crossed with fused males of the same stock. His line was continued from those fraternities in which all diploid offspring were females, non-fused and fused in equal numbers. Triple alleles, xa, xb, xc, were maintained by his system of mating according to the following scheme:

Odd generations $\dfrac{xa+}{xb\ fu}$ ♀ × $xc\ fu$ ♂

Even generations $\dfrac{xa+}{xc\ fu}$ ♀ × $xb\ fu$ ♂

According to these formulae all zygous offspring would be heterozygous and hence female but in every generation a few two-allele fraternities appeared showing diploid males always associated with sex-linkage of fused.

If sex-differentiation were shifted to a second pair of factors $\left(\dfrac{xa + ya}{xa\,fu\;yb}\;♀ \times xa\,fu\,ya\;♂\right)$, diploid males should appear without sex-linkage of fused. Failure to obtain this condition despite prolonged selection proves that only one allelic series was involved in Bostian's experiment.

Whiting (1940) showed quadruple alleles in two stocks. Fused was similarly sex-linked in each stock, indicating that a single series was involved. Matings of heterozygous females with fused males within each stock resulted in two-allele fraternities with zygous males as well as females in proportions indicating linkage: ♀♀ + 9, fu 1, ♂♂ + 1, fu 9 or ♀♀ + 1, fu 9, ♂♂ + 9, fu 1. In crosses between the stocks the linkage was masked since all zygous offspring were female, non-fused and fused in equal numbers, as is characteristic of three-allele fraternities.

Dr. Whiting now has nine sex alleles in five stocks. Linkage with fused is shown in closecrosses (two-allele) but is masked in outcrosses (three-allele) except that stocks xa/xb and xa/xi have one sex allele in common. Diploid males, xa/xa, appear in half the crosses between these two stocks.

Dr. Whiting's data, showing that the sex-differentiating pairs of his five stocks are fused-linked, indicates that a single pair or series is involved in sex-differentiation within each stock. His stocks might conceivably be identical in x, all xa/xb, but differ in other factors. This is extremely unlikely, however, in view of the high probability of obtaining homozygosis of x with consequent non-sex-linkage of fused, as would be expected if y, for example, were the sex-differentiator, $\dfrac{xa + ya}{xa\,fu\;yb}$.

Backcross Experiment Involving Fused

Two stocks were supplied by Dr. Whiting, marked by different non-allelic recessive eye colors and each having the sex-linked female-sterile gene fused, which was maintained by crossing heterozygous females, $+/fu$, to fused males, fu.   For the experiment orange-eyed xa/xb females heterozygous for fused, $\dfrac{o\ xa\ +}{o\ xb\ fu}$, of one stock were crossed with red-eyed xe or xf fused males of the other stock and the reciprocal was also made.   The F_1 black-eyed females and the haploid males, orange or red following their maternal eye color, are fused and non-fused in approximately equal numbers.   No diploid (black-eyed) males occur in F_1.

Ninety-seven black-eyed fused F_2 males from the reciprocal crosses produced a sufficient number of diploids with both P_1 stock females to be included in the summary of data (Table 1).   Forty-eight of these males were from the cross orange females by red fused males, and forty-nine from the reciprocal cross.   Since in the F_1 females heterozygous for fused, the fused came from the P_1 sire, and since the black-eyed fused F_2 males were used for backcrossing, the expectation was that, except for crossovers, these fused males would carry the sex allele from the paternal P_1 stock.   This occurred in 86 of the 97 cases.   The remaining 11 (crossovers) had a maternal P_1 sex allele.   This, a crossover value of 11.3 per cent., is in good agreement with the expectation based on previous crossover values for fused and the sex factor.

Because of the necessity of maintaining the female-sterile gene fused by mating non-fused females to fused males, it is to be expected that the majority of heterozygous stock females will have this factor associated with the same sex allele as that of the 86 non-crossover F_2 fused males.   The two-allele fraternities from these males should then show deficiency of fused daughters and corresponding excess of fused sons.   Such proved to be the case for all the matings of 67 males and for some

TABLE 1

DATA FOR BACKCROSSES OF BLACK-EYED FUSED F₂ MALES FROM RECIPROCAL CROSSES OF ORANGE XA/XB AND RED XE/XF STOCKS

F₂ males	Three-allele fraternities					Two-allele fraternities						
	Females		Haploid males		females / haploid males	Females		Diploid males		Haploid males		females / haploid males
with paternal sex allele.	non-fu	fu	non-fu	fu		non-fu	fu	non-fu	fu	non-fu	fu	
*67	3829	3099	1670	1480	2.53	3830	383	35	464	2000	1690	1.28
†15	706	641	326	279	2.35	114	740	42	12	373	311	1.47
‡4	204	166	77	49	3.01	18	86	9	12	45	35	1.28
						107	20	2	9	54	69	
with maternal sex allele.												
*2	107	68	32	39	2.44	36	8	0	5	12	19	1.65
†8	368	293	141	141	2.86	60	322	16	7	190	165	1.01
‡1	46	45	44	37	1.12	6	18	0	0	3	10	1.21
						26	7	0	4	22	12	

* Males siring deficiency of fused daughters in two-allele fraternities.
† Males siring excess of fused daughters in two-allele fraternities.
‡ Males siring deficiency and excess of fused daughters in different two-allele fraternities. The two types of two-allele fraternities are recorded separately.

of the matings of four others.   The remaining tests of the non-crossover F_2 males showed that crossingover had occurred in the paternal stock so that a minority of females produced fraternities with the reverse type of linkage, excess of fused daughters, deficiency of fused diploid sons.

Since fused in the F_1 females becomes assoicated by crossingover with the sex allele from the maternal stock usually combined with non-fused, the expectation is that excess of fused daughters and of wild-type diploid sons will be sired by the crossover F_2 fused males.   Eight of the eleven crossover males gave this result with all their mates and one with one of his two mates.

Ratio of females to haploid males in the fraternities showing linkage is 1.294 ± 0.055 and it is approximately twice as high, 2.537 ± 0.092, in the fraternities with linkage masked.   This is to be expected, since all the fertilized eggs are female-producing in the latter case, but only half in the former.

The mean ratio of diploid males to their sisters is 0.104 ± 0.007 for the fraternities showing linkage.

The crossover value for fused and x based on the fraternities showing linkage is 10.6 per cent. (674 crossovers, 5,714 non-crossovers).

If the sex factor pair xa/xb linked with fused were the sex differentiator in both stocks and one other pair of sex factors were present, homozygous for different alleles in the two stocks, ya/ya and yb/yb, fraternities with diploid males and showing linkage should be sired by 50 per cent. of the F_2 fused males by paternal stock females and 50 per cent. by maternal stock females. $\left(P_1 \text{ stocks} \right.$ $\dfrac{xa + ya}{xb\, fu\, ya}$ and $\left. \dfrac{xa + yb}{xb\, fu\, yb} \right)$.   Actually the percentages were 88.7 and 11.3.

If the sex factor pair linked with fused were not the sex differentiator (this is, however, contrary to Dr. Whiting's evidence), the non-crossover F_2 fused males

would sire diploid sons when backcrossed to paternal stock females, but not to maternal stock, while the crossover F_2 fused males would sire diploid sons when backcrossed to maternal stock females, but not to paternal stock. But in this case, since the sex differentiator in the stock was not linked to fused, linkage would be masked whether diploid males were produced or not. $\left(P_1 \text{ stocks } \dfrac{xa + ya}{xa\ fu\ yb} \text{ and } \dfrac{xb + ya}{xb\ fu\ yb}\right)$. The data show sex-linkage of fused in all the fraternities with diploid males.

The results obtained in these experiments cannot be interpreted on a basis of more than a single series of allelic factors.

THEORETICAL CONSIDERATIONS

It seems likely that sex is rarely, if ever, differentiated by a single gene. Such may indeed be the first evolutionary step initiating the process, but soon other differences will be accumulated until a differential chromosome segment is developed with many genes linked together into a factor segregating as a unit in meiosis. Thus in *Drosophila melanogaster,* the entire X-chromosome containing numerous but unnumbered sex genes segregates from the Y in spermatogenesis. Crossing-over between the two X's in the female tends to equalize differences, producing uniformity in the population and preventing the evolution of diverse X-chromosome races.

In Lymantria polygenic sex differences have been accumulated distinguishing geographically separated races in each one of which sex balance is normal. There is here a series of homologous Z factors balanced against homologous W factors, but they can not, for the most part, function as alleles in crosses because of intersexuality and consequent sterility of the hybrids. It is possible also that crossingover may occur to some extent in the hybrid males between the Z's of different racial origin so that they do not function as unified alleles.

In species with sex highly differentiated morphologically and even in relatively "undifferentiated" races

there tends to be a dominating unit factor difference, the
retention and evolution of which serves to check the
production of intersexual and sterile types.   The Hy-
menoptera are a highly evolved group of insects showing
wide sex differentiation, both structural and functional.
A multiple factor scheme therefore seems unlikely *a
priori.*

Since the differential maturation hypothesis (to ac-
count for the replacement of diploid males by females)
has now been disproved, the only alternative to multiple
factors would seem to be a series of multiple alleles, dupli-
cating each other in phenotypic effect but consisting basic-
ally of an unknown number of diverse genes.   These must
have been so selected as to have a complementary effect
in female production, this complementary effect being
maintained by haploid parthenogensis subjecting the sex
differentiators (Whiting, 1935) "equally to natural selec-
tion.   They can not, therefore, degenerate by the accumu-
lation of recessive lethals.   Were it not for parthenogene-
sis, the complementary scheme might shift over to one
of simple dominance, the crossing of a heterozygote with
a recessive as in other forms."

Summary

Evidence is presented showing that sex-differentiating
factors in Habrobracon are allelic to each other in a
single series (multiple alleles) rather than occurring in
separately segregating pairs (multiple factors).   Back-
cross breeding tests of the composition of F_2 males proves
that quadruple alleles are involved in the crosses reported
in this paper.

Bostian's proof of triple alleles in his inbreeding
experiment and Whiting's evidence for nine alleles linked
with fused are discussed.

In contrast to a multiple factor hypothesis, the theory
of multiple alleles is regarded as more consistent with
general principles of the evolution of sex differentiation
in the entire group of Hymenoptera.

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REVIEWS AND COMMENTS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Criteria for Vertebrate Subspecies, Species, and Genera. By CHARLES M. BOGERT, W. FRANK BLAIR, EMMETT REID DUNN, E. RAYMOND HALL, CARL L. HUBBS, ERNST MAYR, and GEORGE GAYLORD SIMPSON. Ann. New York Acad. Sci., 44, 1943: 105–188. $1.00.

THE seven papers comprising this symposium bring to notice taxonomic practices and viewpoints employed by workers concerned with each major group of vertebrate animals. Consecutive reading of the items is likely to create a kaleidoscopic effect, for of necessity each contributor in preparing his paper was largely unapprised of the manner of treatment adopted by his associates. But even though organization and emphasis vary greatly among the authors, one is encouraged to find acceptance of the same fundamental concepts of speciation. None seems to question a normal, or at least frequent, continuity in evolution from subspecies to species to genus, nor do any fail to appreciate, at least in a general way, the genetic mechanisms involved in speciation and the necessity for statistical understanding—aspects especially developed by Blair and Simpson, respectively. Divergence comes in shaping definitions, in drawing lines between stages in the evolutionary continuum, and in rating the several criteria that may be utilized. These differences depend largely on whether the worker emphasizes the practical or the theoretical in taxonomy and whether he is willing to declare, rather subjectively, that two geographically isolated forms would interbreed if

thrown together or, contrarily, feels constrained to adhere closely to ascertained facts concerning their morphological divergence or overlap.

In sifting the discussions for points of agreement, we find that the genus is accorded little attention and ordinarily is dismissed as a category of convenience without real boundaries in the system and dependent on uncircumscribable similarities and differences in morphology (Dunn, Hall, Hubbs, Mayr). Simpson's theoretical definition of the genus as a category within which there are genetically discontinuous units does not account for monotypic genera, a few of which at least would seem legitimate. Authors who touch on the question of the size of genera favor large units because they are more useful in showing relationships.

It is agreed that the morphological characters of subspecies, species and genera are not intrinsically different. It is the continuity or discontinuity, genetically and morphologically, of the assemblages of individuals bearing these characters which figures in the definitions.

No one declares in favor of quadrinomials for minor differentiations below the subspecies. As a rule of thumb, differentiation must have proceeded to a point where about 75 per cent. of the animals in a population are morphologically distinguishable from those in adjacent populations before subspecific designations are given (Hubbs, Dunn, Mayr). Hubbs makes special plea for the recognition of lower categories than subspecies, but without nomenclatural treatment. This might profitably be heeded by ornithologists and mammalogists who tend to encumber their taxonomy with very imperfectly differentiated subspecies. However, the ichthyologists' custom of reserving the term "race" for such minor categories should not be pressed upon other workers who for long have used "race" and "subspecies" as synonyms.

Intergradation, the *sine qua non* of the subspecific state to many vertebrate taxonomists, receives much attention. The more penetrating discussions (Hubbs, Dunn) of this

feature commendably acknowledge a wide variety of conditions under this heading. I do not see that most types of intergradation exclude the biologic factor of reproductive continuity, or the possibility of it; thus they are not purely morphological phenomena as Mayr would seem to imply.

Schism among the group of authors is sharpest on the criteria for species. This is plain from Mayr's statement: The species concept based "on the criterion of morphological distinctness or of a gap in morphological characters is invalid. . . . The probability of reproductive isolation is the primary criterion." With this stand Blair would agree, but he would make his test of reproductive isolation through laboratory experiments on fertility. To the reviewer a physiologic isolation in nature is quite as impressive as a failure of crossing of captive animals. Also, I think there is as much chance of breaking down some fertility barriers as there is of breaking certain psychological and other physiological isolations operative in the wild. Should one type of reproductive isolation be viewed as so much more significant than the other? Blair, following the lead of the botanist Turesson, distinguishes cenospecies in the mice of the genus *Peromyscus* in contrast to "incipient species" which are reproductively isolated from one another in nature but prove interfertile in forced matings.

Simpson would agree in principle with Mayr's viewpoint on species, but in practice in dealing with fossil material he is of course forced to follow morphological criteria. Rather pedantically, he distinguishes the biologic or genetic species from the morphological approximation to it that results from the prevailing parallel between morphological differentiation and reproductive isolation. And in turn, he distinguishes the taxonomist's species based on available specimens, which species is but an inference as to the nature of the true morphological species. Simpson's paper, much the most extensive one of the group, performs excellent service in clearly con-

trasting and relating vertical and horizontal species—a discussion well worth the careful attention of all paleontologists.   In defining a genetic species Simpson fails to preclude the subspecies, for he says the species is "a group of organisms so constituted and so situated in nature that a hereditary character of any one of these organisms may be (possibly, but not necessarily) transmitted to a descendant of any other."

Dunn and Hubbs emphasize morphological criteria, but it should be made clear that their position is in no way extreme in this direction.   Dunn states some of the objections to the admittedly logical and attractive criterion of mutual infertility: "it is usually impossible to apply it to the material with which" the taxonomist has to deal; "in many animals actual discontinuity of breeding may occur long before mutual infertility sets in . . . and cases are known in which two populations of what is demonstrably the same species [morphologically?] may manifest actual discontinuity of breeding and act toward each other as two species."

Hubbs perhaps wisely refuses to be bound by any one criterion and he concludes on this vein: "Even when all single tests for the species level break down, a form may be recognized as a species by reason of the usual validity of a series of criteria, just as some subspecies and species may be known by the usual though not invariable possession of each of a series of characters. . . . Neither conventionalized views nor subjective subterfuges—whether by the old-line systematist or by the modern speciationist —can transcend the facts, or create a simple 'correct' system of taxonomy or a simple theory of speciation out of a situation that is inherently complex."

Justice can scarcely be done in review to the many shades of meaning in the statements of the contributing authors.   Each has had a wealth of experience in his field and holds well considered opinions worthy of a hearing. This symposium has served its purpose admirably.— ALDEN H. MILLER.

Systematics, Cytogenetics and Evolution in Crepis. By Ernest
    B. Babcock. Bot. Rev., 8, 1942: 139–190.[1]

With tenacity of purpose Babcock and his associates
have devoted twenty years of work to the study of the
single plant genus *Crepis*.   Although the final monograph
awaits publication, many significant results have already
been presented.   I know of no large genus of animals
that is now as well known, both taxonomically and cyto-
genetically, as *Crepis*.   The recent work of Patterson,
Sturtevant, Dobzhansky and Spencer promises that *Dro-
sophila* may eventually surpass *Crepis* in this respect,
but this goal is still in the far distance.

Crepis forms a compact genus, composed of 196 known
species, which have descended from a common ancestral
stock, probably somewhere in western Asia, and which
have radiated into Africa, Europe, Asia and North
America.   With the help of morphological, ecological and
cytological criteria it can be established rather reliably
which species are primitive and which species belong to a
single line of descent.   This permits the study of evolu-
tionary trends within sub-groups of the genus.

The special advantage of *Crepis* is that it has few chro-
mosomes, which usually can be recognized individually.
The emphasis of Babcock's work is therefore on cyto-
genetics.   The primitive karyotype (chromosome pat-
tern) of the genus consists in the haploid phase of 5 or 6
long, equi-armed chromosomes.   Repeatedly and in inde-
pendent lines this type tends to evolve toward one with
fewer (3 or 4) and shorter chromosomes with uneven
arms.   Parallel, but not necessarily a result, is a change
in morphology and life cycle from large and coarse peren-
nials to small, delicate annuals.   Gene and chromosomal
mutations are common: "It appears that gross structural
changes in the chromosomes have been of evolutionary
importance mainly by producing intraspecific sterility
barriers. . . . Differentiation within and between plant

[1] Supplemented by: Genetic Evolutionary Processes in Crepis.   By E. B.
Babcock, G. L. Stebbins, Jr., and J. A. Jenkins.   Am. Nat., 76, 1942: 337–
363.

species depend mainly, if not wholly, upon gene muta-
tions.'' Sterility is the only isolating factor that is dis-
cussed in detail. A treatment of other mechanisms that
may assist in the prevention of cross fertilization of re-
lated sympatric species would have been of considerable
interest. Or is sterility really the only isolating mecha-
nism in *Crepis?*

Interspecific hybridization is rather frequent, but it is
of secondary importance as a speciation process. Poly-
ploids are few. The ordinary species concept, as derived
from sexual diploids, breaks down in certain groups of
hybridizing polyploids and associated agamic forms.
Apomixis, frequently found in aggressive and wide-
spread forms, leads to temporary advantages, but inevi-
tably to eventual extinction.

The gene contents of not a single species of *Crepis* is
anywhere nearly as well explored as that of *Drosophila
melanogaster, Zea mays,* or *Gossypium.* As far as tax-
onomy is concerned, the degree of perfection which intra-
specific population analysis has reached in fishes, mam-
mals and birds does not seem to be approached in *Crepis.*
Filling this gap will require the statistical analysis of
future mass collections. Still, Professor Babcock can
point out with pride that he accomplished what he had set
out to do: the classification of the species in a large and
difficult genus and the establishment of the principal evo-
lutionary processes that have led to this diversification
of species. The *Crepis* studies represent a major contri-
bution to the study of evolution.—ERNST MAYR.

The Structure and Origin of Species With a Discussion of In-
traspecific Variability and Related Nomenclatural Problems.
By W. H. CAMP AND C. L. GILLY. Brittonia, 4, 1943: 323–385.

PLANT systematists Camp and Gilly, of the New York
Botanical Garden, openly engage the idea that the ''spe-
cies problem'' is a problem of *the* species. Along with
Huxley and certain other modern analysts of speciation
they direct their attack on the different kinds of species.

They recognize a dozen categories, for which, ignoring previously proposed names, they erect the following terms, more or less strictly of Greek origin: homogeneon, phenon, parageneon, dysploidion, euploidion, alloploidion, micton, rheogameon, cleistogameon, heterogameon, and, for species in which apomixis is present, apogameon and agameon. The authors recognize but I believe underestimate the difficulties and disagreements that would arise in the practical application of this classification of species types. Perhaps because they are experienced taxonomists, they do not advocate revolutionary changes in nomenclatorial practice: seemingly they would apply the conventional binomial to all types of species. For routine systematics they favor only two intraspecific categories— the subspecies for those having geographical as well as morphological integrity and the forma for those, with some genetical continuity, which commingle with other types of the species. The less precise category of varietas they discredit, although they think it should probably not be abandoned in exploratory systematics. Strangely, they propose only one new intraspecific grouping, the phenogen, for units of a phenon which are intersterile but which can not be distinguished except by genetic analysis.

Camp and Gilly also propose a taxonomy for types of systematic research, stressing the newer approaches without discrediting routine or exploratory systematics. To more critical analyses unaccompanied by cytological or genetic analysis they restrict, I think unwisely, the term "general systematics." For taxonomic research involving cytology and genetics they provide a new designation, biosystematy. They regard this as *the* critical phase of systematics by which origins, relationships and taxonomic status are objectively demonstrable. I have found, on the contrary, that uncertainties in interpretation, particularly as to group ranking, are often accentuated by more extended and refined research. It seems to me that the prime value of experimental systematics lies in the revealing of speciational processes, and that

such processes are inherently so diverse and complex as to fit very poorly into fixed concepts of group ranking and nomenclature.

The references to recent treatises on the philosophy of plant systematics, and the critical remarks thereon, will prove of particular value to students of speciation.

The Biotic Provinces of North America. By LEE R. DICE. Ann Arbor: University of Michigan Press, 1943: i–viii, 1–78, Map 1. $1.75.

MERRIAM's ''life zones'' were long popular among students of geographical distribution, but are now commonly displaced by other areal units. In current studies the biotic divisions of North America are stressed. Dice's ''biotic provinces'' in particular override the life zones or belts. The general environment rather than temperature is thus stressed as the controlling factor in the natural, correlated distribution of plants and animals.

The boundaries of the biotic provinces from the transcontinental Eskimoan and Hudsonian in the north to the mosaic of units in the southwestern United States and in northern and central Mexico are admittedly generalized, but I think Dice understates the indefiniteness of many boundary lines when he writes ''the facts of distribution would in some situations be almost as well satisfied if the boundary between two adjacent biotic provinces were moved ten, or, more rarely, as much as fifty, miles.'' The provinces as mapped are very broadly generalized, with little interdigitation and with intermixtures eliminated by definition. Perhaps this simplification of boundaries is the greatest fault of the new classification, as contrasted with the intricate pattern of life zones as mapped by Merriam's followers.

Without citation of evidence fresh-water communities are held to be included in the biotic provinces. In general, however, river systems are much more significant in the limitation of fresh-water faunas than are the boundaries of forests and other major vegetational types on

which the biotic provinces are largely based. In some places, however, the distribution of fishes transcends the limits of river systems to form major patterns somewhat corresponding with the recognized biotic provinces, particularly the Hudsonian, Canadian, and Austroriparian. The essential distinction of the southeastern and southwestern biotas of the United States is well confirmed by the distribution of fishes.

This latest contribution to regional biogeography, though scholarly in treatment, strong and modern in ecological concepts and thorough in historical considerations, shares with earlier general studies an emphasis on the distribution of large, conspicuous and "higher" organisms and maintains an approach that is essentially one of impression and opinion. How and where the boundaries between biotic provinces are to be drawn, which biotic areas are to be ranked as provinces rather than as districts or other subdivisions, how the provinces in turn are to be organized in larger units (which Dice makes no effort to do), and even whether the distributional patterns of various groups of animals and plants coincide sufficiently well to justify the recognition and mapping of general biotic provinces, are questions which I think can not be answered adequately until a very extensive accumulation of data has been subjected to thorough and critical statistical analyses.

Notices of New Books

Wildlife Refuges. By Ira N. Gabrielson. New York: Macmillan Co., 1943: i–xiii, 1–257, figs. 1–17, pls. 1–32. $4.00. An accomplished naturalist as well as America's foremost wildlife administrator, Ira N. Gabrielson presents this detailed discussion of wildlife refuges as a sequel to his excellent book on "Wildlife Conservation" (1941). Under the initial leadership of Jay N. Darling and the succeeding administration of Dr. Gabrielson, and the immediate supervision of J. Clark Salyer, II, the national wildlife refuge system has become an effective force of preservation and restoration. As made clear in this excellent book, it is not only the game birds and mammals that are being

protected, but all forms of life. The refuge operations are gratifying examples of successfully applied ecology. As such they are contributing much to biology, particularly to the biology of populations, a subject of current interest to students of evolution.

Biochemistry and Morphogenesis. By JOSEPH NEEDHAM. Cambridge: at The University Press; New York: The Macmillan Co., 1942: i–xvi, 1–787, 328 illustrations. $12.50.—"Superb," "colossal" and other attributives ordinarily usurped by the motion-picture industry could rightly be applied to Needham's "Biochemistry and Morphogenesis." This book is a scholarly, masterful compendium and synthesis of the published information on the physiology of development, with particular reference to the chemical background. No one who is investigating the fundamental basis of morphogenesis can afford to be without this book. And when it is recalled that many leaders in biological thought have predicted that this is the field in which greatest progress is due in biology, the vast importance of Needham's work will be apparent.

Studies of Evaporation and Transpiration under Controlled Conditions. By EMMET MARTIN. Carnegie Inst. Wash. Publ. 550, 1943: i–iii, 1–48, figs. 1–17. $0.40 (paper bound, offset printing).—This contribution to plant physiology contains a discussion of ecological applications.

Seven Papers in Genetics and Physiological Genetics of Drosophila melanogaster. By RICHARD BLANC, WERNER BRAUN, ELDON J. GARDNER, RICHARD GOLDSCHMIDT, CLAUDE A. VILLEE, JR. Univ. Calif. Publ. Zool., 49, 1942: 1–183, pls. 1–11, 18 figs. $2.00. —This series of papers from Goldschmidt's productive laboratory further analyzes "the concept of gene action on the basis of relative rates, times, and thresholds of reaction." The first, by Richard Blanc, reporting "Observations on the Production of Wing Scalloping in Drosophila melanogaster," supports Goldschmidt's explanation rather than "Waddington's more complicated hypothesis." The same author with Werner Braun then treats "Phenocopies and X Radiation in Drosophila melanogaster," lining up the production of these mutation-simulating "morphoses" or "roentgenmorphoses" with the current rate theory

of development; statistical treatment is emphasized. Then Blanc, in collaboration with Claude A. Villee, Jr., deals with "The Effect of X Radiation upon Bristle Formation in Drosophila melanogaster," from much the same point of view. Werner Braun follows with "The Effect of Changes in the Time of Development on the Phenotype Mutants of Drosophila melanogaster." Eldon F. Gardner contributes "A Further Study of Genetic Modification of Dominance, Especially by Position Effects." The viewpoints of penetrance and expressivity are featured in Goldschmidt and Gardner's article, "A Further Contribution to the Analysis of Scalloped Wings in Drosophila melanogaster."

The final and longest contribution, by Claude A. Villee, Jr., is "A Study of Hereditary Homoeosis: the Mutant Tetraltera in Drosophila melanogaster." This investigation bears on problems of regeneration and homology as well as genetics. Certain phenotypes of the tetraltera mutant were found to duplicate almost exactly, in wing structure, the aberrant dipteran *Termitoxenia*.

Tertiary Prairie Grasses and Other Herbs from the High Plains. By MAXIM K. ELIAS. Special Papers, Geol. Soc. Am., 41, 1942; 1–176, pls. 1–17, fig. 1. $1.50.—Students of grasslands as well as paleobotanists and stratigraphers will find much of interest in this account of the origin or incursion and the subsequent evolution of the vegetation of the Great Plains. Students of evolution will also find material of significance, for the "comparative study of the fossil and living forms reveals evolutionary trends of the seeds of prairie grasses. The rather small and generalized Miocene ancestor gave rise to greatly diversified Pliocene and Recent species. The seeds of these include small and very large, very slender, and very stout forms, all of them variously adapted for protection against drought and for more efficient dispersal." Like many other paleontologists, Elias stresses the direct effect of the environment, and evolutionary trends which are not necessarily connected with minor fluctuating changes in the environments. A distinctly modern view is that interspecific hybridization has resulted "occasionally in stable mutations, subspecies or even new species (amphidiploids)." It is pointed out that such reticulate evolution complicates the phylogenetic picture. The family tree of the Stipeae is offered in a "spirit of symbolic interpretation." A brief discussion of "Taxonomy in Relation to Evolution" is included.

The Birds of Southern Veracruz, Mexico. By Alexander Wetmore. Proc. U. S. Nat. Mus., 93, 1943: 215–340, pls. 26–28, fig. 11.—Although largely an annotated list of the birds, this paper contributes information on the general ecology of a little-known part of Middle America. The higher sections of two mountains in the Sierra de Tuxtla are placed in the Subtropical rather than the Humid or Arid division of the Tropical Zone, and "the Subtropical element here must be considered a remnant or fragment from the cooler climatic conditions of the Pleistocene." The relicts, however, are of tropical-mountain rather than northern derivation.

Biological Results of the Last Cruise of the Carnegie. By Herbert W. Graham and others. Carnegie Inst. Wash. Publ. 555, 1943: i–v, 1–92, 69 figs., 4 maps. $1.00 (paper), $1.50 (cloth).—Graham's paper on The Phytoplankton is probably the item of most general interest, certainly the most important oceanographical contribution, in the series of twelve reports.

SHORTER ARTICLES AND DISCUSSION

POPULATION STRUCTURE IN TOADS[1]

In the last decade it has become increasingly apparent that
the development of diversity within species populations is in-
timately concerned with such factors as size of breeding popu-
lations, periodic fluctuation of population size, sex ratio, activity
range and differential survival of progeny. The sum total of
these factors, population structure, determines to a great extent
the relative importance of the roles which mutation, natural selec-
tion and random fluctuation of gene frequency play in the genetic
configuration of the species.

It is obviously desirable to secure adequate knowledge of popu-
lation structure in a variety of living species representative of
the animal and plant kingdoms. At present such comparative
data are woefully lacking. It seems, therefore, worthwhile to
place on record some pertinent data on population structure in
toads. These data, referring chiefly to *Bufo americanus*, were
collected by the writer in northeastern Oklahoma in the spring
of 1941. The area investigated constitutes, roughly, a 75-mile
east-west transect extending from Sand Springs, Oklahoma, to
Kansas, Oklahoma. This transect includes portions of three
of the biotic districts of Oklahoma as defined by W. F. Blair
and Hubbell (1938): Osage Savanna (western 1/5 of transect),
Cherokee Prairie (middle 7/15 of transect), and Ozark (eastern
1/3 of transect). In the eastern one-third *B. americanus* occurs
with *B. fowleri;* in the western two-thirds it occurs with *B.
woodhousii.*

Observations on more than 50 ponds and pools from March 22
to May 26 showed 767 male *B. americanus* and 40 female *B.
americanus.* None of these is a duplicate observation of the same
individual, since all animals were marked by toe clipping when
first captured. These figures are somewhat low for females,
since they do not call and are less conspicuous in the ponds.
In a number of instances, however, ponds were cleared of all
evident females at night and then checked in the morning to

see how many egg masses had been deposited. Since females almost always oviposit the same night on which they come to the pond, the number of egg masses found in the morning is a rather accurate count of the female toads which were overlooked the night before plus such females as may have come to the pond after the survey. Results indicate that some females may be overlooked, the number depending upon the nature of the pool, but there is no indication that the sex ratio in the breeding pools is 1:1. Collections of non-breeding toads, however, show more nearly the expected 1:1 ratio. Evidently, then, females do not go to the breeding pools each year, and many of the calling males find no mates. Wright (1914) found that male *B. americanus* far outnumbered females in the breeding ponds around Ithaca, N. Y. In central Oklahoma Bragg (1940) found that male *B. woodhousii* outnumbered females in breeding congresses and concluded that females do not breed each year.

Because of the incompleteness of isolating mechanisms mixed breeding aggregations occur. Table 1 indicates all aggregations where both male and female toads were present (A refers to *B. americanus* and W to *B. woodhousii*). There is considerable opportunity for hybridization between *B. americanus* and *B. woodhousii*. No male *B. fowleri* was found in any of the *B. americanus* aggregations. The experience of the writer in northeastern Oklahoma indicates, however, that straggler males of *B. americanus* may occur in breeding populations of *B. fowleri*. No opportunity for studying this overlap was afforded during the present study. Because of the most severe flood on record or other causes there was no breeding of *B. fowleri* in the area under observation in 1941; observations throughout the summer showed a complete absence of tadpoles and young toads of this species.

The average size of breeding aggregations of *B. americanus* in the area under consideration is small. Reference to Table 1 shows that in only three out of 15 aggregations where both male and female *B. americanus* were found in the ponds were there more than 30 toads present. The average size of 15 aggregations was 19.3 toads (16.6 males and 2.6 females). In the area studied the breeding sites utilized by *B. americanus* are for the most part brook pools and temporary rain pools. With the exception of a limited number of flood plain sloughs the region contains few or no large natural pools which might be used for breeding. The largest breeding aggregation observed was in an artificial pond

north of Locust Grove, and it seems likely that the largest breeding populations are to be found in bodies of water impounded by man. At Ithaca, N. Y., the breeding congresses of *B. americanus* are evidently quite large, as Wright (1914) states that it is not a rare observation "to find a thousand toads in one small pond." The writer's observations (Blair, 1941) on *B. americanus* in Indiana indicate breeding populations intermediate in size between the small aggregations of northeastern Oklahoma and the very large ones of New York.

TABLE 1

COMPOSITION OF ALL BREEDING AGGREGATIONS (BOTH MALE AND FEMALE TOADS PRESENT) FOUND IN NORTHEASTERN OKLAHOMA IN 1941.

A refers to *B. americanus* and W to *B. woodhousii* ; ♂A × ♀A indicates a clasping pair.

Locality	Type pool	Date	Toads present				
			♂A	♀A	♂A ×♀A	♂W	♀W
Locust Grove, Okla.	Shallow slough	March 22	10		1		
Locust Grove, Okla.	Large spring-fed pond	March 22	63	2	8		
Kansas, Okla.	Brook pool	March 23	9		1		
Kansas, Okla.	Brook pool	March 23	13	1	4		
Locust Grove, Okla.	Brook pool	March 31	13		1		
Sand Springs, Okla.	Pond	April 2	14	1	1		
Locust Grove, Okla.	Ditch puddle	April 15	2		1		
Scraper, Okla.	Ditch puddle	April 16	15		3		
Scraper, Okla.	Brook pool	April 16	12		2		
Kansas, Okla.	Brook pool	April 16	28	2	5		
Locust Grove, Okla.	Pool near creek	April 25	1			1	1
Kansas, Okla.	Brook pool	April 26	31	1			
Tulsa, Okla.	Brook pool	April 30	5				1
Tulsa, Okla.	Oil slush pond	April 30	2			4	1
Tulsa, Okla.	Pond	May 1	3	1		2	
Tulsa, Okla.	Rain pool	May 5	2			11	1
Tulsa, Okla.	Rain pool	May 7				8	1
Sand Springs, Okla.	Pond	May 20	3	1		1	
Locust Grove, Okla.	Large spring-fed pond	May 21	2		1		
Salina, Okla.	Backwater of creek	May 26	3	3			

Male toads which do not find mates may continue calling for some time. The following data for calling male *B. americanus* recaptured after being marked in the breeding pond give the number of days (numeral in parentheses) after being marked that the toad was retaken, and the number of toads (numeral outside of parentheses) retaken after a given number of days: (2)7, (3)2, (6)7, (7)3, (8)2, (9)8, (10)2, (11)2, (15)17, (17)1, (20)2, (22)1, (24)3, (26)1, (33)3, (34)2, (41)3, (42)1 and (51)1. Such records do not imply continuous nightly calling over the period indicated; unmated toads which have ceased calling may resume calling on the advent of rainfall.

The male chorus daily loses and gains members. For instance, 14 male *B. woodhousii* and one male *B. americanus* were calling

see how many egg masses had been deposited. Since females almost always oviposit the same night on which they come to the pond, the number of egg masses found in the morning is a rather accurate count of the female toads which were overlooked the night before plus such females as may have come to the pond after the survey. Results indicate that some females may be overlooked, the number depending upon the nature of the pool, but there is no indication that the sex ratio in the breeding pools is 1 : 1. Collections of non-breeding toads, however, show more nearly the expected 1 : 1 ratio. Evidently, then, females do not go to the breeding pools each year, and many of the calling males find no mates. Wright (**1914**) found that male *B. americanus* far outnumbered females in the breeding ponds around Ithaca, N. Y. In central Oklahoma Bragg (**1940**) found that male *B. woodhousii* outnumbered females in breeding congresses and concluded that females do not breed each year.

Because of the incompleteness of isolating mechanisms mixed breeding aggregations occur. Table 1 indicates all aggregations where both male and female toads were present (A refers to *B. americanus* and W to *B. woodhousii*). There is considerable opportunity for hybridization between *B. americanus* and *B. woodhousii*. No male *B. fowleri* was found in any of the *B. americanus* aggregations. The experience of the writer in northeastern Oklahoma indicates, however, that straggler males of *B. americanus* may occur in breeding populations of *B. fowleri*. No opportunity for studying this overlap was afforded during the present study. Because of the most severe flood on record or other causes there was no breeding of *B. fowleri* in the area under observation in 1941; observations throughout the summer showed a complete absence of tadpoles and young toads of this species.

The average size of breeding aggregations of *B. americanus* in the area under consideration is small. Reference to Table 1 shows that in only three out of 15 aggregations where both male and female *B. americanus* were found in the ponds were there more than 30 toads present. The average size of 15 aggregations was 19.3 toads (16.6 males and 2.6 females). In the area studied the breeding sites utilized by *B. americanus* are for the most part brook pools and temporary rain pools. With the exception of a limited number of flood plain sloughs the region contains few or no large natural pools which might be used for breeding. The largest breeding aggregation observed was in an artificial pond

north of Locust Grove, and it seems likely that the largest breeding populations are to be found in bodies of water impounded by man. At Ithaca, N. Y., the breeding congresses of *B. americanus* are evidently quite large, as Wright (1914) states that it is not a rare observation "to find a thousand toads in one small pond." The writer's observations (Blair, 1941) on *B. americanus* in Indiana indicate breeding populations intermediate in size between the small aggregations of northeastern Oklahoma and the very large ones of New York.

TABLE 1

Composition of All Breeding Aggregations (Both Male and Female Toads Present) Found in Northeastern Oklahoma in 1941.

A refers to *B. americanus* and W to *B. woodhousii* ; $\male A \times \female A$ indicates a clasping pair.

Locality	Type pool	Date	Toads present				
			$\male A$	$\female A$	$\male A \times \female A$	$\male W$	$\female W$
Locust Grove, Okla.	Shallow slough	March 22	10		1		
Locust Grove, Okla.	Large spring-fed pond	March 22	63	2	8		
Kansas, Okla.	Brook pool	March 23	9		1		
Kansas, Okla.	Brook pool	March 23	13	1	4		
Locust Grove, Okla.	Brook pool	March 31	13		1		
Sand Springs, Okla.	Pond	April 2	14	1	1		
Locust Grove, Okla.	Ditch puddle	April 15	2		1		
Scraper, Okla.	Ditch puddle	April 16	15		3		
Scraper, Okla.	Brook pool	April 16	12		2		
Kansas, Okla.	Brook pool	April 16	28	2	5		
Locust Grove, Okla.	Pool near creek	April 25	1			1	1
Kansas, Okla.	Brook pool	April 26	31		1		
Tulsa, Okla.	Brook pool	April 30	5				1
Tulsa, Okla.	Oil slush pond	April 30	2			4	1
Tulsa, Okla.	Pond	May 1	3	1		2	
Tulsa, Okla.	Rain pool	May 5	2			11	1
Tulsa, Okla.	Rain pool	May 7				8	1
Sand Springs, Okla.	Pond	May 20	3	1		1	
Locust Grove, Okla.	Large spring-fed pond	May 21	2		1		
Salina, Okla.	Backwater of creek	May 26	3	3			

Male toads which do not find mates may continue calling for some time. The following data for calling male *B. americanus* recaptured after being marked in the breeding pond give the number of days (numeral in parentheses) after being marked that the toad was retaken, and the number of toads (numeral outside of parentheses) retaken after a given number of days: (2)7, (3)2, (6)7, (7)3, (8)2, (9)8, (10)2, (11)2, (15)17, (17)1, (20)2, (22)1, (24)3, (26)1, (33)3, (34)2, (41)3, (42)1 and (51)1. Such records do not imply continuous nightly calling over the period indicated; unmated toads which have ceased calling may resume calling on the advent of rainfall.

The male chorus daily loses and gains members. For instance, 14 male *B. woodhousii* and one male *B. americanus* were calling

in a slough near Grand River on the night of April 25; of 13 male
B. woodhousii and five male *B. americanus* calling in the same
slough two nights later, seven *B. woodhousii* and one *B. ameri-*
canus were carry-overs from the first date.   Of 18 male *B. ameri-*
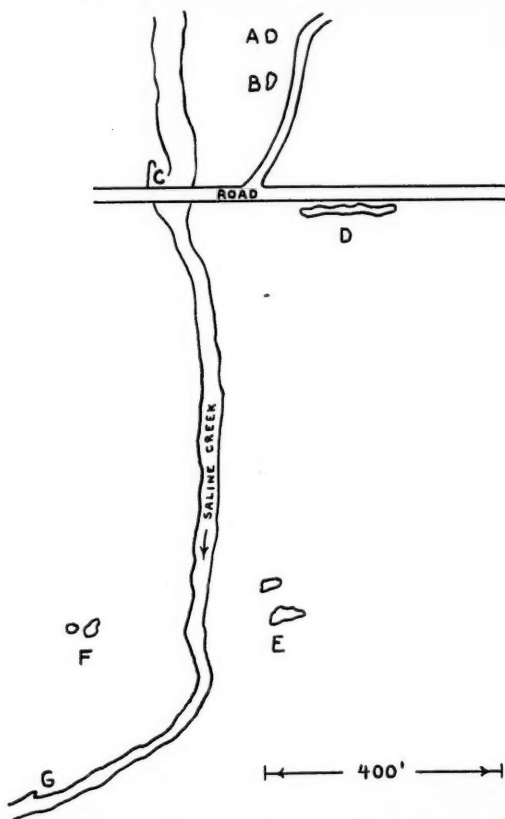
Fig. 1.   Inter-pond migration of male *B. americanus;* see text for details.

canus calling in a ditch puddle southeast of Locust Grove on
April 15, two were calling in a chorus of nine male *B. americanus*
in the same puddle fifteen days earlier.   The detailed history of a
small area along Saline Creek, in the Ozark district, is given in
the following compilation (see Fig. 1) :

March 31.   Nine male *B. americanus* calling at A.
April   15.   Of seven male *B. americanus* calling at A, two were marked at
                   A March 31; of five male *B. americanus* calling at B, two were

marked at A March 31; of four male *B. americanus* calling at
D, one was marked at A March 31.

April 17. Severe local flood covers area with 10–15 feet of water for
several days.

May 3. Of two male *B. americanus* calling at A (pool about half
filled with sand after flood), one was marked at A March 31.

May 11. Of 16 male *B. americanus* calling at E, one was marked at A
March 31; of seven male *B. americanus* calling at F, two were
marked at A March 31 and one at A April 15.

May 18. Of three male *B. americanus* calling at C, one was marked at
F May 11.

May 21. Of five male *B. americanus* calling at E, one was marked at
A March 31.

May 26. Of three calling male *B. americanus* and three female *B. ameri-
canus* at G, none was previously marked.

It is evident from this data that once arrived at a given pool
male toads do not always remain until mated or until cessation
of the reproductive urge leaves them unmated. The following
tabulation lists male toads captured at breeding sites other than
those where they were marked originally; the straight-line dis-
tance traveled and the time elapsed since marking are indicated:

| | *Bufo americanus* | | | | *Bufo americanus* (cont.) | |
	Distance (feet)	Time (days)			Distance (feet)	Time (days)
1.	45	15		13.	1,050	41
2.	45	15		14.	1,050	26
3.	350	15		15.	1,800	10
4.	390	33				
5.	400	42			*Bufo fowleri*	
6.	640	15		1.	250	9
7.	640	24		2.	250	12
8.	660	33		3.	260	3
9.	780	15		4.	300	3
10.	900	7		5.	660	7
11.	1,000	41		6.	810	8
12.	1,050	41		7.	1,100	8

In seven of these cases (*B. americanus* Nos. 7, 8, 10, 11, 12, 13
and 14) there was the possibility of passive movement by flood
waters; the end achieved is the same, however. Two of the *B.
americanus* (Nos. 6 and 9) must have gone by routes more than
twice as long as indicated or else have crossed two ponds with a
combined width of more than 400 feet. *Bufo fowleri* No. 7 had
to swim the Illinois River, a stream 50–200 feet wide with a strong
current at the narrower portions, to reach the point where it was
recaptured. Such records indicate a considerable movement of

males from pond to pond during the breeding season. Piatt (1941) marked 120 mating pairs and 18 non-mating toads of *B. americanus* at four breeding sites of a 10-acre pond at Rensselaerville, New York; he recaptured only one toad, a female, at a breeding site different than that where marked.

Summary

(1) Data on population structure in toads, chiefly *Bufo americanus*, were gathered in northeastern Oklahoma in the spring of 1941.

(2) In the area studied, *B. americanus* males greatly outnumbered females in the breeding ponds (6:1 considering all breeding aggregations where both male and female toads were present).

(3) Mixed aggregations of *B. americanus* and *B. woodhousii* occur.

(4) The average size of 15 breeding aggregations of *B. americanus* was found to be 19.3 toads (16.6 males and 2.6 females).

(5) The male chorus daily loses and gains members. Male toads which do not find mates may continue calling in the same pond or may migrate to another pond and call there.

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A HORMONE-PRODUCED TAXONOMIC CHARACTER IN PLATYPOECILUS MACULATUS DIAGNOSTIC OF WILD P. XIPHIDIUM[1]

THE adult males of *Platypoecilus xiphidium* Hubbs and Gordon of the Rio Soto la Marina in northeastern Mexico are distinguished from all other male members of their genus by a small, swordlike prolongation of several ventral rays of their caudal fins. The specific name of this platyfish was designed to emphasize this point. In a "key" to the four known species of *Platypoecilus*, the caudal sword may be used as a taxonomic character to separate *P. xiphidium* populations from those of *P. couchianus* (Girard) of the Rio San Juan System, *P. variatus* Meek of the Rio Panuco System and *P. maculatus* Günther of the Jamapa, Papaloapan, Coatzacoalcos and Usumacinta River systems. A brief survey of the speciation problems in the platyfish-swordtail group has been presented recently by Gordon (1943).

In the course of study of the effects of sex hormones upon developing sex characters and upon the courtship behavior of *P. maculatus*, a number of striking deviations from the normal were found by H. Cohen (1942). This discussion is concerned with the changes noted in the development of the caudal fin which were brought about by the use of the synthetic steroid, pregneninolone.[2]

For the purposes of these hormone studies it was held essential to use immature fish of known initial, genetic, sex. Since the external secondary sex characters in the platyfishes are not developed until approaching maturity (frequently a period of six to eight months) a genetic method for the determination of the sexes in the newly born was employed.

A black-spotted female *P. maculatus* of the constitution (W) + (Z)Sp, (New York Zoological Society Culture Number 8A–1), was mated with an unspotted male, (Z) + (Z) + (Culture Number 23–11). Ninety-six of their offspring were reared to maturity under normal conditions, that is, without the use of hor-

mones: 40 were spotted and males; 56 were unspotted and females. Deviations from these expected results are relatively infrequent, for according to Fraser and Gordon (1929) the cross-over value is about one per cent. (Parenthetically, it may be stated that endocrinologists who use fishes as their test animals may profit by the use of species in which sex may be determined early, either by genetic methods or by some early differentiating sexually dimorphic structures like those which Turner (1941) found in the anal fins of juvenile *Gambusia affinis*).

As part of these studies, fifteen unspotted, two-weeks-old, genetically determined, female *P. maculatus* of the same parents as indicated above were placed in three three-gallon aquaria. The water temperature was maintained at approximately 24° C. The fish were fed the usual types of food: dried shrimp, dried liver meal mixture and live tubifex worms. Every week they received, in addition, 5 mgm of pregneninolone crystals, some of which the fish appeared to swallow with the particles of their food. The hormone was given over a period of five months. The control fish received the identical food and were maintained under similar conditions.

The effects upon the pregneninolone-treated females were quite marked and were expressed in the changes seen in the gonads, muscles and various parts of the skeleton. The anal fin, unmodified in the normal female, was transformed into a male-like gonopodium (confirming the work of Grobstein, 1940, 1941) and this organ was supported by a typical male-like gonopodial suspensorium and instrumented by a gonopodal muscle. The courtship behavior was typically male-like. In addition to all these effects of the androgenic hormone, a change in the structure of the ventral rays of the caudal fin was induced which is the main subject of this discussion.

The caudal fin of normal male and female *P. maculatus* is symmetrical. The caudal fin of pregneninolone-treated females is asymmetrical: Some of the fin rays of the ventral sector are much shorter, while others are much longer than their counterparts in the dorsal sector. Specifically, rays number 7, 8 and 9 of the caudal fin, counting from the ventral to the dorsal region, are abnormally short, while ray number 6 is considerably longer than its dorsal counterpart. The shortening of some rays and the lengthening of another produces the appearance of the "sword" in the experimentally masculinized female *P. maculatus*. This

tail structure resembles the normal sword of the male *P. xiphidium.*

The platyfishes are closely allied to the swordtails of the genus *Xiphophorus* (see Hubbs and Gordon, 1943). The swordtails have "swords" of varying sizes: the male *X. hellerii* Heckel has the longest, about the length of its body; that of *X. montezumae* Jordan and Snyder is about half its body; while that of *X. pygmaeus* Hubbs and Gordon is tiny, shorter than that of *P xiphidium,* which is about as long as the width of its eye.

Female swordtails normally carry no swords, yet some, that have been known to produce offspring in their prime, develop short swords in their old age. Swords have been induced in young females by treatment with androgenic hormones, and in one case by estrones; for a detailed discussion of the problem the reader is referred to Witschi (1942). Furthermore, it has long been known that female swordtails carry hereditary factors for sword formation because they transmit these factors to their intergeneric hybrid sons when they are mated to male platyfish. It is possible to find among the many domesticated, aquarium-reared stocks of the platyfishes individuals which meet all the taxonomic criteria for *P. maculatus* except that some of the males may have a short sword, evidence of a promiscuous mating with the swordtail in the past. A genetic test for *X. hellerii* genes in aquarium stocks of *P. maculatus* has been briefly outlined by Gordon (1942).

The above leads us to postulate that it is likely that platyfishes, as well as swordtails, have genetic factors for sword formation. The genic complex for this structure varies with the species and with the sex of the species involved. These underlying complexes reflect the over-all genetic differences between the species. The normal manifestation of the hereditary factors for long sword, short sword or no sword at all are subject to changes under the influence of exogenous agents, specifically by treatment with unusual doses of sex hormones. Under these conditions, swords may be induced in female *X. hellerii* or in female *P. maculatus;* others of the group have not yet been tested.

In studies to discover the genetic nature of the differences between, and similarities in, the species of the platyfish-swordtail group some significant facts have been reported by Gordon (1943) concerning the distribution of a number of genes and their frequencies. In the light of this work and related details, the arti-

ficial transformation of a structure in one species of a xiphophorin fish to simulate a "key" character of another species is important only in that the experiment brings to light the basic genetic factors common to the group as a whole.

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INDEX

573